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(71) Applicant *(for all designated States except US)*: SYNAPTIC PHARMACEUTICAL CORPORATION [US/US]; 215 College Road, Paramus, NJ 07652 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GERALD, Christophe, P., G. [FR/US]; 204-B Union Street, Ridgewood, NJ 07450 (US). WEINSHANK, Richard, L. [US/US]; 268 Vandelinda Avenue, Teaneck, NJ 07666 (US). WALKER, Mary, W. [US/US]; 9 Spruce Street, Elmwood Park, NJ 07407 (US). BRANCHEK, Theresa [US/US]; 518 Standish Road, Teaneck, NJ 07666 (US).

(74) Agent: WHITE, John, P.; Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, NY 10036 (US).

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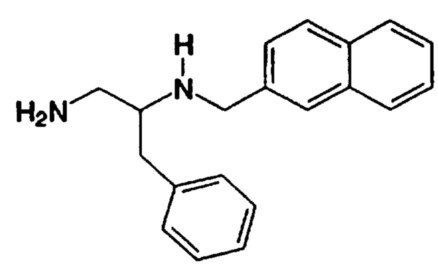

(I)

(57) Abstract

The invention provides methods of modifying feeding behavior, including increasing or decreasing food consumption, e.g., in connection with treating obesity, bulimia or anorexia. These methods involve administration of compounds that are selective agonists or antagonists for the Y5 receptor. One such compound has structure (I). In addition, this invention provides an isolated nucleic acid molecule encoding a Y5 receptor, an isolated Y5 receptor protein, vectors comprising an isolated nucleic acid molecule encoding a Y5 receptor, cells comprising such vectors, antibodies directed to the Y5 receptor, nucleic acid probes useful for detecting nucleic acid encoding Y5 receptors, antisense aligonucleotides complementary to any unique sequences of a nucleic acid molecule which encodes a Y5 receptor, and nonhuman transgenic animals which express DNA encoding a normal or a mutant Y5 receptor.

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METHODS OF MODIFYING FEEDING BEHAVIOR, COMPOUNDS USEFUL IN SUCH METHODS, AND DNA ENCODING A HYPOTHALAMIC ATYPICAL NEUROPEPTIDE Y/PEPTIDE YY RECEPTOR (Y5)

Background of the Invention

Throughout this application, various publications are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citations for these publications may be found at the end of the specification, preceding the sequence listing and the claims.

Neuropeptide Y (NPY) is a member of the pancreatic polypeptide family with widespread distribution throughout the mammalian nervous system. NPY and its relatives (peptide YY or PYY, and pancreatic polypeptide or PP) elicit a broad range of physiological effects through activation of at least five G protein-coupled receptor subtypes known as Y1, Y2, Y3, Y4 (or PP), and the "atypical Y1". The role of NPY as the most powerful stimulant of feeding behavior yet described is thought to occur primarily through activation of the hypothalamic "atypical Y1" receptor. This receptor is unique in that its classification was based solely on feeding behavior data, rather than radioligand binding data, unlike the Y1, Y2, Y3, and Y4 (or PP) receptors, each of which were described previously in both radioligand binding and functional assays.

The peptide neurotransmitter neuropeptide Y (NPY) is a 36 amino acid member of the pancreatic polypeptide family with widespread distribution throughout the mammalian nervous system. NPY is considered to be the most powerful stimulant of feeding behavior yet described (Clark et al., 1984; Levine and Morley, 1984; Stanley and Leibowitz, 1984). Direct injection into the hypothalamus

of satiated rats, for example, can increase food intake up to 10-fold over a 4-hour period (Stanley et al., 1992). The role of NPY in normal and abnormal eating behavior, and the ability to interfere with NPY-dependent pathways as a means to appetite and weight control, are areas of great interest in pharmacological and pharmaceutical research (Sahu and Kalra, 1993; Dryden et al., 1994). Any credible means of studying or controlling NPY-dependent feeding behavior, however, must necessarily be highly specific as NPY can act through at least 5 pharmacologically defined receptor subtypes to elicit a wide variety of physiological functions (Dumont et al., 1992). It is therefore vital that knowledge of the molecular biology and structural diversity of the individual receptor subtypes be understood as part of a rational drug design approach to develop subtype selective compounds. A brief review of NPY receptor pharmacology is summarized below and also in Table 1.

TABLE 1: Pharmacologically defined receptors for NPY and related pancreatic polypeptides.

Rank orders of affinity for key peptides (NPY, PYY, PP, [Leu^{31},Pro^{34}]NPY, NPY_{2-36}, and NPY_{13-36}) are based on previously reported binding and functional data (Schwartz et al., 1990; Wahlestedt et al., 1991; Dumont et al., 1992; Wahlestedt and Reis, 1993). Data for the Y2 receptor were disclosed in PCT International Application No. PCT/US95/01469, filed February 3, 1995, International Publication No. WO 95/21245, published August 10, 1995 the foregoing contents of which are hereby incorporated by reference. Data for the Y4 receptor were disclosed in PCT International Application No. PCT/US94/14436 filed December 28, 1994, International Publication No. WO 95/17906, published August 10, 1995 the contents of which are hereby incorporated by reference. Missing peptides in the series reflect a lack of published information.

Table 1 reflects current information obtained with cloned human Y1, Y2, Y4, and Y5 receptors.

TABLE 1

Receptor	Affinity (pK$_i$ or pEC$_{50}$)					
	11 to 10	10 to 9	9 to 8	8 to 7	7 to 6	< 6
Y1	NPY PYY [Leu^{31},Pro^{34}]NPY		NPY$_{2-36}$	NPY$_{13-36}$	PP	
Y2		PYY NPY NPY$_{2-36}$	NPY$_{13-36}$			[Leu^{31}, Pro^{34}] NPY PP
Y3		NPY	[Pro^{34}] NPY	NPY$_{13-36}$ PP		PYY
Y4	PP			PYY [Leu^{31}, Pro^{34}]-NPY NPY	NPY$_{2-36}$	NPY$_{13-36}$
Y5 or atypical Y1 (feeding)			PYY NPY NPY$_{2-36}$ [Leu^{31}, Pro^{34}] NPY	NPY$_{13-36}$ D-Trp^{32}NPY		

NPY Receptor Pharmacology

NPY receptor pharmacology has historically been based on structure/activity relationships within the pancreatic polypeptide family. The entire family includes the namesake pancreatic polypeptide (PP), synthesized primarily by endocrine cells in the pancreas; peptide YY (PYY), synthesized primarily by endocrine cells in the gut; and NPY, synthesized primarily in neurons (Michel, 1991; Dumont et al., 1992; Wahlestedt and Reis, 1993). All pancreatic polypeptide family members share a compact

structure involving a "PP-fold" and a conserved C-terminal hexapeptide ending in Tyr^{36} (or Y^{36} in the single letter code). The striking conservation of Y^{36} has prompted the reference to the pancreatic polypeptides' receptors as "Y-type" receptors (Wahlestedt et al., 1987), all of which are proposed to function as seven transmembrane-spanning G protein-coupled receptors (Dumont et al., 1992).

The Y1 receptor recognizes NPY $\geq$ PYY >> PP (Grundemar et al., 1992). The receptor requires both the N- and the C-terminal regions of the peptides for optimal recognition. Exchange of Gln^{34} in NPY or PYY with the analogous residue from PP (Pro^{34}), however, is well-tolerated. The Y1 receptor has been cloned from a variety of species including human, rat and mouse (Larhammar et al, 1992; Herzog et al, 1992; Eva et al, 1990; Eva et al, 1992). The Y2 receptor recognizes PYY ~ NPY >> PP and is relatively tolerant of N-terminal deletion (Grundemar et al., 1992). The receptor has a strict requirement for structure in the C-terminus (Arg^{33}-Gln^{34}-Arg^{35}-Tyr^{36}-NH_2); exchange of Gln^{34} with Pro^{34}, as in PP, is not well tolerated. The Y2 receptor has recently been cloned. The Y3 receptor is characterized by a strong preference for NPY over PYY and PP (Wahlestedt et al., 1991). [Pro^{34}]NPY is reasonably well tolerated even though PP, which also contains Pro^{34}, does not bind well to the Y3 receptor. The Y3 receptor (Y3) has not yet been cloned. The Y4 receptor binds PP > PYY > NPY. Like the Y1, the Y4 requires both the N- and the C-terminal regions of the peptides for optimal recognition. The "atypical Y1" or "feeding" receptor was defined exclusively by injection of several pancreatic polypeptide analogs into the paraventricular nucleus of the rat hypothalamus which stimulated feeding behavior with the following rank order: $NPY_{2-36} \geq$ NPY ~ PYY ~ [Leu^{31},Pro^{34}]NPY > NPY_{13-36} (Kalra et al., 1991; Stanley et al., 1992). The profile is

similar to that of a Y1-like receptor except for the anomalous ability of NPY_{2-36} to stimulate food intake with potency equivalent or better than that of NPY. A subsequent report in *J. Med. Chem.* by Balasubramaniam et al. (1994) showed that feeding can be regulated by [D-Trp^{32}]NPY. While this peptide was presented as an NPY antagonist, the published data at least in part support a stimulatory effect of [D-Trp^{32}]NPY on feeding. [D-Trp^{32}]NPY thereby represents another diagnostic tool for receptor identification. In contrast to other NPY receptor subtypes, the "feeding" receptor has never been characterized for peptide binding affinity in radioligand binding assays and the fact that a single receptor could be responsible for the feeding response has been impossible to validate in the absence of an isolated receptor protein; the possibility exists, for example, that the feeding response could be a composite profile of Y1 and Y2 subtypes.

This invention now reports the isolation by expression cloning of a novel Y-type receptor from a rat hypothalamic cDNA library, along with its pharmacological characterization, *in situ* localization, and human homolog. The data provided link this newly-cloned receptor subtype, from now on referred to as the Y5 subtype, to the "atypical Y1" feeding response. This discovery therefore provides a novel approach, through the use of heterologous expression systems, to develop a subtype selective antagonist for obesity and other indications.

This invention is based on the use of a ^{125}I-PYY-based expression cloning technique to isolate a rat hypothalamic cDNA encoding an "atypical Y1" receptor referred to herein as the Y5 receptor subtype. This application also concerns the isolation and characterization of a Y5 homolog from human hippocampus.

Protein sequence analysis reveals that the Y5 receptor belongs to the G protein- coupled receptor superfamily. Both the human and rat homolog display $\leq$ 42% identity in transmembrane domains with the previously cloned "Y-type" receptors. Rat brain localization studies using *in situ* hybridization techniques verified the existence of Y5 receptor mRNA in rat hypothalamus. Pharmacological evaluation revealed the following similarities between the Y5 and the "atypical Y1" receptor. 1) Peptides bound to the Y5 receptor with a rank order of potency identical to that described for the feeding response: NPY $\geq$ NPY_{2-36} = PYY = [Leu^{31}, Pro^{34}]NPY >> NPY_{13-36}. 2) The Y5 receptor was negatively coupled to cAMP accumulation, as had been proposed for the "atypical Y1" receptor. 3) Peptides activated the Y5 receptor with a rank order of potency identical to that described for the feeding response. 4) The reported feeding "modulator" [D-Trp^{32}]NPY bound selectively to the Y5 receptor and subsequently activated the receptor. 5) Both the Y5 and the "atypical Y1" receptors were sensitive to deletions or modifications in the midregion of NPY and related peptide ligands. These data support the identity of the Y5 receptor as the previously described "atypical Y1", and furthermore indicate a role for the Y5 receptor as a potential target in the treatment of obesity, metabolism, and appetite disorders.

The treatment of disorders or diseases associated with the inhibition of the Y5 receptor subtype, especially diseases caused by eating disorders like obesity, bulimia nervosa, diabetes, dislipidimia, may be effected by administration of compounds which bind selectively to the Y5 receptor and inhibit the activation of the Y5 receptor. Furthermore, any disease states in which the Y5 receptor subtype is involved, for example, memory loss, epileptic seizures, migraine, sleep disturbance, pain, and affective disorders such as depression and

anxiety may also be treated using compounds which bind selectively to the Y5 receptor.

Summary of the Invention

This invention provides a method of modifying a subject's feeding behavior which comprises administering to the subject a compound which is a Y5 receptor agonist or antagonist in an amount effective to alter the subject's consumption of food and thereby modify the subject's feeding behavior.

This invention also provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the binding of the compound to the human receptor is characterized by a K_i less than 100 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount.

Additionally, this invention provides a method of treating a subject's feeding disorder which comprises administering to the subject a peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the compound's binding to the human Y5 receptor is characterized by a K_i less than 10 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount.

This invention further provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by a K_i less than 100 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount; and (b) the binding of the compound to any other

human Y-type receptor is characterized by a K_i greater than 1000 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount.

This invention also provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by a K_i less than 1 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount; and (b) the compound's binding to any other human Y-type receptor is characterized by a K_i greater than 100 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount.

This invention further provides a method of treating a subject's feeding disorder which comprises administering to the subject a peptidyl compound which is a Y5 receptor agonist effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by a K_i less than 1 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount; and (b) the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 25 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount.

This invention provides a method of treating a subject's feeding disorder which comprises administering to the subject a peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by a K_i less than 0.1 nanomolar when measured in the presence

of ^{125}I-PYY in a predetermined amount; and (b) the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 1 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount.

This invention further provides a method of treating a subject's feeding disorder which comprises administering to the subject a peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by a K_i less than 0.01 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount; and (b) the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 1 nanomolar when measured in the presence of ^{125}I-PYY in a predetermined amount.

Additionally, this invention provides an isolated nucleic acid encoding a Y5 receptor. This invention also provides an isolated Y5 receptor protein. This invention provides a vector comprising the above-described nucleic acid.

This invention also provides a plasmid which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to the DNA encoding the human Y5 receptor as to permit expression thereof designated pcEXV-hY5 (ATCC Accession No. 75943). This invention further provides a plasmid which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to the DNA encoding the rat Y5 receptor as to permit expression thereof designated pcEXV-rY5 (ATCC Accession No. 75944).

This invention provides a mammalian cell comprising the

above-described plasmid or vector.

This invention also provides a nucleic acid probe comprising a nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid encoding a Y5 receptor.

Additionally, this invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a Y5 receptor so as to prevent translation of the mRNA.

This invention also provides an antibody directed to a Y5 receptor.

This invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to reduce activity of a human Y5 receptor by passing through a cell membrane and binding specifically with mRNA encoding a human Y5 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

This invention also provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a human Y5 receptor and a pharmaceutically acceptable carrier. This invention further provides a pharmaceutical composition comprising an amount of an agonist effective to increase activity of a Y5 receptor and a pharmaceutically acceptable carrier. This invention further provides the above-described pharmaceutical composition which comprises an amount of an antibody effective to block binding of a ligand to the Y5 receptor and a pharmaceutically acceptable carrier.

This invention additionally provides a transgenic

nonhuman mammal expressing DNA encoding a human Y5 receptor.

This invention also provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises contacting a plurality of cells transfected with and expressing DNA encoding the Y5 receptor, or a membrane fraction from a cell extract of such cells, with the ligand under conditions permitting binding of ligands to such receptor, detecting the presence of any such ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand specifically binds to the Y5 receptor.

This invention further provides a method for determining whether a ligand is a Y5 receptor agonist which comprises contacting a cell transfected with and expressing nucleic acid encoding a human Y5 receptor with the ligand under conditions permitting activation of the Y5 receptor, detecting an increase in Y5 receptor activity, and thereby determining whether the ligand is a human Y5 receptor agonist.

This invention provides a method for determining whether a ligand is a Y5 receptor antagonist which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor with the ligand in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of the Y5 receptor, detecting a decrease in Y5 receptor activity, and thereby determining whether the ligand is a Y5 receptor antagonist.

This invention further provides a method of screening a plurality of chemical compounds not known to bind to a Y5 receptor to identify a compound which specifically binds to the Y5 receptor, which comprises (a) contacting a cell

transfected with and expressing DNA encoding the Y5 receptor, or a membrane fraction from a cell extract of such cells, with a compound known to bind specifically to the Y5 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the Y5 receptor, under conditions permitting binding of compounds known to bind the Y5 receptor; (c) determining whether the binding of the compound known to bind to the Y5 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of compounds; and if so (d) separately determining the binding to the Y5 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the Y5 receptor.

This invention also provides a method of screening a plurality of chemical compounds not known to activate a Y5 receptor to identify a compound which activates the Y5 receptor which comprises (a) contacting a cell transfected with and expressing the Y5 receptor, or a membrane fraction from a cell extract of such cells, with the plurality of compounds not known to bind specifically to the Y5 receptor, under conditions permitting activation of the Y5 receptor; (b) determining whether the activity of the Y5 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the Y5 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the Y5 receptor.

This invention further provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a Y5 receptor to identify a compound which inhibits the activation of the Y5 receptor, which comprises (a) contacting a cell transfected with and

expressing the Y5 receptor, or a membrane fraction from a cell extract of such cells, with the plurality of compounds in the presence of a known Y5 receptor agonist, under conditions permitting activation of the Y5 receptor; (b) determining whether the activation of the Y5 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the Y5 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the Y5 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the Y5 receptor.

Additionally, this invention provides a process for identifying a chemical compound which specifically binds to a Y5 receptor, which comprises contacting nonneuronal cells expressing on their cell surface the Y5 receptor, or a membrane fraction from a cell extract of such cells, with the chemical compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the Y5 receptor.

This invention also provides a process involving competitive binding for identifying a chemical compound which specifically binds to a Y5 receptor which comprises separately contacting nonneuronal cells expressing on their cell surface a Y5 receptor, or a membrane fraction from a cell extract of such cells, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the Y5 receptor, a decrease in the binding of the second chemical compound to the Y5 receptor in the presence of the chemical compound indicating that the chemical compound binds to the Y5 receptor.

This invention further provides a process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a Y5 receptor, or a membrane fraction from a cell extract of such cells, with the chemical compound under conditions suitable for activation of the Y5 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in second messenger response in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor.

This invention also provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a Y5 receptor, or a membrane fraction from a cell extract of such cells, with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound indicating that the chemical compound inhibits activation of the Y5 receptor.

This invention additionally provides a method of treating a subject's abnormality, wherein the abnormality is alleviated by the inhibition of a Y5 receptor which comprises administering to a subject an effective amount of Y5 receptor antagonist. This invention also provides

a method of treating a subject's abnormality wherein the abnormality is alleviated by the activation of a Y5 receptor which comprises administering to a subject an effective amount of a Y5 receptor agonist.

This invention further provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific allelic form of a human Y5 receptor which comprises: a. obtaining DNA from a subject to be tested; digesting the DNA with restriction enzymes; c. separating the resulting DNA fragments; d. contacting the fragments with a detectably labeled nucleic acid probe capable of specifically hybridizing with a sequence uniquely present within the sequence of a nucleic acid molecule encoding the allelic form of the human Y5 receptor; and e. detecting the presence of labeled probe from the subject to be tested, the presence of such hybridized probe indicating that the subject is predisposed to the disorder.

This invention also provides a method of preparing the isolated Y5 receptor which comprises: a. inserting nucleic acid encoding Y5 receptor in a suitable vector which comprises the regulatory elements necessary for expression of the nucleic acid operatively linked to the nucleic acid encoding a Y5 receptor; b. inserting the resulting vector in a suitable host cell so as to obtain a cell which produces the Y5 receptor; c. recovering the receptor produced by the resulting cell; and d. purifying the receptor so recovered.

Brief Description of the Figures

Figure 1 Competitive displacement of ^{125}I-PYY on membranes from rat hypothalamus. Membranes were incubated with ^{125}I-PYY and increasing concentrations of peptide competitors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis. Data are representative of at least two independent experiments. IC_{50} values for these compounds are listed separately in Table 2.

Figure 2 Competitive displacement of ^{125}I-PYY_{3-36} on membranes from rat hypothalamus. Membranes were incubated with ^{125}I-PYY_{3-36} and increasing concentrations of peptide competitors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis. Data are representative of at least two independent experiments. IC_{50} values for these compounds are listed separately in Table 2.

Figure 3 Nucleotide sequence of the rat hypothalamic Y5 cDNA clone (Seq. I.D. No 1). Initiation and stop codons are underlined. Only partial 5′ and 3′ untranslated sequences are shown.

Figure 4 Corresponding amino acid sequence of the rat hypothalamic Y5 cDNA clone (Seq. I.D. No. 2).

Figure 5 Nucleotide sequence of the human hippocampal Y5 cDNA clone (Seq. I.D. No. 3). Initiation and stop codons are underlined. Only partial 5′ and 3′ untranslated sequences are shown.

Figure 6 Corresponding amino acid sequence of the human hippocampal Y5 cDNA clone(Seq. I.D. No. 4).

Figure 7 A-E. Comparison of coding nucleotide sequences

between rat hypothalamic Y5 (top row) and human hippocampal Y5 (bottom row) cDNA clones (84.1% nucleotide identity). F-G. Comparison of deduced amino acid sequences between rat hypothalamic Y5 (top row) and human hippocampal Y5 (bottom row) cDNA clones (87.2% overall and 98.8% transmembrane domain identities).

Figure 8 Comparison of the human Y5 receptor deduced amino acid sequence with those of the human Y1, Y2, Y4 sequences. Solid bars, the seven putative membrane-spanning domains (TM I-VII). Shading, identities between receptor sequences.

Figure 9 Equilibrium binding of ^{125}I-PYY to membranes from COS-7 cells transiently expressing rat Y5 receptors. Membranes were incubated with ^{125}I-PYY for the times indicated, in the presence or absence of 300 nM human NPY. Specific binding, B, was plotted against time, t, to obtain the maximum number of equilibrium binding sites, B_{max}, and observed association rate, K_{obs}, according to the equation, $B = B_{max} * (1 - e^{-(kobs * t)})$. Binding is shown as the percentage of total equilibrium binding, B_{max}, determined by nonlinear regression analysis. Each point represents a triplicate determination.

Figure 10 Saturable equilibrium binding of ^{125}I-PYY to membranes from COS-7 cells transiently expressing rat Y5 receptors. Membranes were incubated with ^{125}I-PYY ranging in concentration from 0.4 pM to 2.7 nM, in the presence or absence of 300 nM human NPY. Specific binding, B, was plotted against the free ^{125}I-PYY concentration, [L], to obtain the maximum number of saturable binding sites, B_{max}, and the ^{125}I-PYY equilibrium dissociation constant, K_d, according to the binding isotherm, $B = B_{max}[L]/([L] + K_d)$. Specific binding is shown. Data are representative of three independent experiments, with each point measured in triplicate.

<u>Figure 11</u> Competitive displacement of ^{125}I-PYY from COS-7 cells transiently expressing rat Y5 receptors. Membranes were incubated with ^{125}I-PYY and increasing concentrations of peptide competitors. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the equation, $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ^{125}I-PYY concentration and K_d is the equilibrium dissociation constant of ^{125}I-PYY. Data are representative of at least two independent experiments. Rank orders of affinity for these and other compounds are listed separately in Table 4.

<u>Figure 12</u> Inhibition of forskolin-stimulated cAMP accumulation in intact 293 cells stably expressing rat Y5 receptors. Functional data were derived from radioimmunoassay of cAMP in 293 cells stimulated with 10 μM forskolin over a 5 minute period. Rat/human NPY was tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 μM over the same period. The EC_{50} value corresponding to 50% maximal activity was determined by nonlinear regression analysis. The data shown are representative of three independent experiments.

<u>Figure 13</u> Schematic diagrams of coronal sections through the rat brain, illustrating the distribution of NPY Y5 receptor mRNA, as visualized microscopically in sections dipped in liquid emulsion. The sections are arranged from rostral (A) to caudal (H). Differences in silver grain density over individual neurons in a given area are indicated by the hatching gradient. The full definitions for the abbreviations are as follows:

Aco = anterior cortical amygdaloid nucleus;
AD = anterodorsal thalamic nucleus;
APT = anterior pretectal nucleus;
Arc = arcuate hypothalamic nucleus;

BLA = basolateral amygdaloid nucleus anterior;
CA3 = field CA3 of Ammon's horn, hippocampus;
CeA = central amygdaloid nucleus;
Cg = cingulate cortex;
CL = centrolateral thalamic nucleus;
CM = central medial thalamic nucleus
DG = dentate gyrus, hippocampus;
DMH = dorsomedial hypothalamic nucleus;
DR = dorsal raphe;
GiA = gigantocellular reticular nucleus, alpha;
HDB = nucleus horizontal limb diagonal band;
InG = intermediate gray layer superior colliculus;
LC = locus coeruleus;
LH = lateral hypothalamic area;
MePV = medial amygdaloid nucleus, posteroventral;
MVe = medial vestibular nucleus;
MHb = medial habenular nucleus;
MPN = medial preoptic nucleus;
PAG = periaqueductal gray;
PaS = parasubiculum;
PC = paracentral thalamic nucleus;
PCRtA = parvocellular reticular nucleus, alpha;
Pe = periventricular hypothalamic nucleus;
PrS = presubiculum;
PN = pontine nuclei;
PVH = paraventricular hypothalamic nucleus;
PVHmp = paraventricular hypothalamic nucleus, medial parvicellular part
PVT = paraventricular thalamic nucleus;
Re = reunions thalamic nucleus;
RLi = rostral linear nucleus raphe;
RSG = retrosplenial cortex;
SCN = suprachiasmatic nucleus;
SNc = substantia nigra, pars compacta; and
SON = supraoptic nucleus.

Figure 14 Partial Nucleotide sequence of the canine Y5 cDNA clone beginning immediately upstream of TM III to the stop codon (underlined). (Seq. I.D. No 5). Only partial untranslated sequences are shown.

Figure 15 Corresponding partial amino acid sequence of the canine Y5 cDNA clone (Seq. I.D. No. 6).

Figure 16 A. Northern blot analysis of various rat tissues. B. Northern blot analysis of various human brain areas: amygdala, caudate nucleus, corpus callosum, hippocampus, whole brain, substantia nigra, subthalamic nucleus, and thalamus. C. Northern blot analysis of various additional human brain areas: cerebellum, cerebral cortex, medulla, spinal cord, occipital lobe, frontal lobe, temporal lobe, and putamen. Hybridization was done under conditions of high stringency, as described in Experimental Details.

Figure 17 Southern blot analysis of human(A) or rat(B) genomic DNA encoding the Y5 receptor subtype. Hybridization was done under conditions of high stringency, as described in Experimental Details.

Figure 18 Time course for equilibrium binding of ^{125}I-Leu^{31},Pro^{34}-PYY to the rat Y5 receptor. Membranes were incubated with 0.08 nM radioligand at room temperature for the length of time indicated in binding buffer containing either 10 mM Na+ or 138 mM Na+.

Figure 19 Guanine Nucleotide Modulation of Y5 Peptide Binding. Human or rat Y5 receptors transiently expressed in COS-7 cell membranes, or human Y5 receptors stably expressed in LM(tk-) cell membranes, were incubated with 0.08 nM ^{125}I-PYY and increasing concentrations of Gpp(NH)p as indicated under standard binding assay conditions. Radioligand binding is reported as cpm, efficiency = 0.8.

For the human Y5 in LM(tk-) (0.007 mg membrane protein/sample), the maximum Δ cpm = -2343. Given a specific activity of 2200 Ci/mmol, the change in radioligand binding is therefore calculated to be -0.6 fmol/0.007 mg protein = -85 fmol/mg membrane protein.

Figure 20 NPY-Dependent Inhibition of Forskolin Stimulated cAMP Accumulation by Cloned Y5 Receptors. Intact cells stably transfected with human or rat Y5 receptors were incubated with forskolin plus a range of human NPY concentrations as indicated. A representative experiment is shown for each receptor system ($n \geq 2$).

Figure 21 Calcium Mobilization: Fura-2 Assay. Cloned human Y-type receptors in the host cells indicated were screened for intracellular calcium mobilization in response to NPY and related peptides. Representative calcium transients are shown for each receptor system.

A. Human Y1 receptor
B. Human Y2 receptor
C. Human Y4 receptor
D. Human Y5 receptor

Figure 22 Structures of Y5-selective compounds. The structures of the compounds evaluated at the human Y-type receptors are given.

Figure 23 Nucleotide sequence of the canine Y5 cDNA clone (Seq. I.D. No. 13). Initiation and stop codons are underlined. Only partial 5′ and 3′ untranslated sequences are shown.

Figure 24 Corresponding amino acid sequence of the canine Y5 cDNA clone (Seq. I.D. No. 14).

Figure 25 Schematic representation of the human Y1/Y5

locus on chromosome 4q. Open boxes represent non-coding exons. Closed boxes indicate coding regions (CDS). Arrows on top of exons 1A, 1B and 1C show transcription starts for the three known alternative splice variants of the Y1 mRNA (Ball, et al., 1995). Arrows under the coding regions show opposite transcriptional directions for the Y1 and Y5 genes. "P*" indicates a PstI restriction site polymorphism described previously in the Y1 locus (Herzog, et al., 1993).

Detailed Description of the Invention

Throughout this application, the following standard abbreviations are used to indicate specific nucleotide bases:

C=cytosine	A=adenine
T=thymine	G=guanine

Furthermore, the term "agonist" is used throughout this application to indicate any peptide or non-peptidyl compound which increases the activity of any of the receptors of the subject invention. The term "antagonist" is used throughout this application to indicate any peptide or non-peptidyl compound which decreases or inhibits the activity of any of the receptors of the subject invention.

The activity of a G-protein coupled receptor such as a Y5 receptor may be measured using any of a variety of appropriate functional assays in which activation of the receptor in question results in an observable change in the level of some second messenger system, including but not limited to adenylate cyclase, calcium mobilization, inositol phospholipid hydrolysis or guanylyl cyclase.

This invention provides a method of modifying a subject's feeding behavior which comprises administering to the subject a compound which is a Y5 receptor agonist or antagonist in an amount effective to alter the subject's consumption of food and thereby modify the subject's feeding behavior. In one embodiment, the compound is a Y5 receptor antagonist and the amount is effective to decrease the consumption of food by the subject. In a further embodiment, the compound is administered in combination with food. In another embodiment the compound is a Y5 receptor agonist and the amount is effective to increase the consumption of food by the

subject. In a further embodiment the compound is administered in combination with food. The subject may be a vertebrate, a mammal, a human or a canine subject.

This invention also provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the binding of the compound to the human Y5 receptor is characterized by a K_i less than 100 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In one embodiment the compound has a K_i less than 50 nanomolar. In another embodiment, the compound has a K_i less than 10 nanomolar. In a further embodiment, the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 10 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In another embodiment, the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 50 nanomolar. In another embodiment, the binding of the compound is characterized by a K_i greater than 100 nanomolar. In one embodiment, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor. In a further embodiment the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to each of the human Y1, human Y2 and human Y4 receptors. The feeding disorder may be obesity or bulimia. The subject may be a vertebrate, a mammal, a human or a canine subject.

This invention further provides a method of treating a subject's feeding disorder which comprises administering to the subject a peptidyl compound which is a Y5 receptor

antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the compound's binding to the human Y5 receptor is characterized by a K_i less than 10 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In one embodiment, the compound's binding is characterized by a K_i less than 1 nanomolar. In another embodiment, the compound's binding to any other human Y-type receptor is characterized by a K_i greater than 10 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In another embodiment the compound's binding to each of the human Y1, human Y2, and human Y4 receptors is characterized by a K_i greater than 10 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In a further embodiment, the compound's binding to any other human Y-type receptor is characterized by a K_i greater than 50 nanomolar. In another embodiment the compound's binding to any other human Y-type receptor is characterized by a K_i greater than 100 nanomolar. In one embodiment, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor. In another embodiment, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to each of the human Y1, human Y2, and human Y4 receptors. The feeding disorder may be obesity or bulimia. The subject may be a vertebrate, a mammal, a human, or a canine subject.

This invention provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by

a K_i less than 100 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration; and (b) the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 1000 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In one embodiment, the binding of the compound to the human Y5 receptor is characterized by a K_i less than 10 nanomolar.

This invention also provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by a K_i less than 1 nanomolar when measured in the presence in ^{125}I-PYY; and (b) the compound's binding to any other human Y-type receptor is characterized by a K_i greater than 100 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In one embodiment, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor. In another embodiment, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to each of the human Y1, human Y2, and human Y4 receptors. The feeding disorder may be anorexia. The subject may be a vertebrate, a mammal, a human, or a canine subject.

This invention further provides a method of treating a subject's feeding disorder which comprises administering to the subject a peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by

a K_i less than 1 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration; and (b) the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 25 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration.

This invention provides a method of treating a subject's feeding disorder which comprises administering to the subject a peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by a K_i less than 0.1 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration; and (b) the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 1 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In one embodiment, the binding of the agonist to any other human Y-type receptor is characterized by a K_i greater than 10 nanomolar.

This invention provides a method of treating a subject's feeding disorder which comprises administering to the subject a peptidyl compound which is a Y5 receptor agonist in an amount effective to increase the activity of the subject's Y5 receptor, wherein (a) the binding of the compound to the human Y5 receptor is characterized by a K_i less than 0.01 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration; and (b) the binding of the compound to any other human Y-type receptor is characterized by a K_i greater than 1 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In one embodiment, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor.

In another embodiment, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to each of the human Y1, human Y2, and human Y4 receptors. In one embodiment, the feeding disorder is anorexia. The subject may be a vertebrate, a mammal, a human, or a canine subject.

In addition, this invention provides an isolated nucleic acid encoding a Y5 receptor. In one embodiment, the Y5 receptor is a vertebrate or a mammalian Y5 receptor. In another embodiment, the Y5 receptor is a human Y5 receptor. In a further embodiment, the isolated nucleic acid encodes a receptor being characterized by an amino acid sequence in the transmembrane region, wherein the amino acid sequence has 60% homology or higher to the amino acid sequence in the transmembrane region of the human Y5 receptor shown in Figure 6. In another embodiment, the Y5 receptor has substantially the same amino acid sequence as described in Figure 4. In another embodiment, the Y5 receptor has substantially the same amino acid sequence as described in Figure 6. In another embodiment, the Y5 receptor has substantially the same amino acid sequence as described in Figure 24.

This invention provides the above-described isolated nucleic acid, wherein the nucleic acid is a DNA. In an embodiment, the DNA is a cDNA. In another embodiment, the DNA is a genomic DNA. In still another embodiment, the nucleic acid is RNA. In a separate embodiment, the nucleic acid encodes a human Y5 receptor. In an embodiment, the human Y5 receptor has the amino acid sequence as described in Figure 6. In another embodiment, the nucleic acid encodes a rat Y5 receptor. In an embodiment, the rat Y5 receptor has the amino acid sequence as shown in Figure 4. In another embodiment, the nucleic acid encodes a canine Y5 receptor. In an

embodiment, the canine Y5 receptor has the amino acid sequence shown in Figure 24.

This invention further provides DNA which is degenerate with any of the DNA shown in Figures 3, 5, 14 and 23, wherein the DNA encodes Y5 receptors having the amino acid sequences shown in Figures 4, 6, 15 and 24, respectively.

This invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of Y5 receptor, but which should not produce phenotypic changes. Alternatively, this invention also encompasses DNAs and cDNAs which hybridize to the DNA, RNA, and cDNA of the subject invention. Hybridization methods are well known to those of skill in the art.

The nucleic acid of the subject invention also includes nucleic acid coding for polypeptide analogs, fragments or derivatives of antigenic polypeptides which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These nucleic acids include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate nucleic acid sequences that facilitate construction of readily expressed vectors.

The nucleic acids described and claimed herein are useful

for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The nucleic acid is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

In a separate embodiment, the nucleic acid encodes a rat Y5 receptor. In another embodiment, the rat Y5 receptor has the amino acid sequence shown in Figure 4.

This invention also provides an isolated Y5 receptor protein. In one embodiment, the Y5 receptor protein is a human Y5 receptor protein. In another embodiment, the human Y5 receptor protein has the amino acid sequence as shown in Figure 6. In a further embodiment, the Y5 receptor protein is a rat Y5 receptor protein. In another embodiment, the rat Y5 receptor protein has the amino acid sequence as shown in Figure 4. In another embodiment, the Y5 receptor protein is a canine Y5 receptor protein. In a further embodiment, the canine Y5 receptor protein has the amino acid sequence as shown in Figure 24.

This invention provides a vector comprising the above-described nucleic acid.

Vectors which comprise the isolated nucleic acid described hereinabove also are provided. Suitable vectors comprise, but are not limited to, a plasmid or a virus. These vectors may be transformed into a suitable host cell to form a host cell vector system for the production of a polypeptide having the biological activity of a Y5 receptor.

This invention provides the above-described vector adapted for expression in a cell which further comprises the regulatory elements necessary for expression of the nucleic acid in the cell operatively linked to the nucleic acid encoding the Y5 receptor as to permit expression thereof. In an embodiment, the cell is a *Xenopus* cell such as an oocyte or melanophore.

This invention provides the above-described vector adapted for expression in a bacterial cell which further comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding the Y5 receptor as to permit expression thereof.

This invention provides the above-described vector adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding the Y5 receptor as to permit expression thereof.

This invention provides the above-described vector adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the Y5 receptor as to permit expression thereof.

In an embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the mammalian Y5 receptor as to permit expression thereof.

In an embodiment, the vector is adapted for expression in

a mammalian cell which comprises the regulatory elements necessary for expression of the nucleica acid in the mammalian cell operatively linked to the nucleic acid encoding the canine Y5 receptor as to permit expression thereof.

In a further embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the human Y5 receptor as to permit expression thereof.

In a still further embodiment, the plasmid is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the rat Y5 receptor as to permit expression thereof.

In a still further embodiment, the plasmid is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding the canine Y5 receptor as to permit expression thereof.

This invention provides the above-described plasmid adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of nucleic acid in a mammalian cell operatively linked to the nucleic acid encoding the mammalian Y5 receptor as to permit expression thereof.

This invention provides a plasmid which comprises the regulatory elements necessary for expression of nucleic acid in a mammalian cell operatively linked to the

nucleic acid encoding the human Y5 receptor as to permit expression thereof designated pcEXV-hY5 (ATCC Accession No. 75943).

This plasmid (pcEXV-hY5) was deposited on November 4, 1994 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 75943.

This invention provides a plasmid which comprises the regulatory elements necessary for expression of nucleic acid in a mammalian cell operatively linked to the nucleic acid encoding the rat Y5 receptor as to permit expression thereof designated pcEXV-rY5 (ATCC Accession No. 75944).

This plasmid (pcEXV-rY5) was deposited on November 4, 1994 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. CRL 75944.

This invention provides a plasmid designated Y5-bd-5 (ATCC Accession No. 97355). This invention also provides a plasmid designated Y5-bd-8 (ATCC Accession No. 97354). These plasmids were deposited on December 1, 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This invention further provides a plasmid designated cY5-

BO11, which comprises a canine Y5 receptor. This plasmid was deposited on May 29, 1996 with the ATCC under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent procedure, and was accorded ATCC Accession No. 97587.

This invention provides a baculovirus designated hY5-BB3 (ATCC Accession No. VR-2520). This baculovirus was deposited on November 15, 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. VR-2520.

This invention provides a mammalian cell comprising the above-described plasmid or vector. In an embodiment, the mammalian cell is a COS-7 cell, a Chinese hamster ovary (CHO) cell, or a neuronal cell such as the glial cell line C6.

In another embodiment, the mammalian cell is a 293 human embryonic kidney cell designated 293-rY5-14 (ATCC Accession No. CRL 11757). This cell (293-rY5-14) was deposited on November 4, 1994 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. CRL 11757.

In a further embodiment, the mammalian cell is a mouse fibroblast LM(tk-) cell, containing the plasmid pcEXV-hY5 and designated L-hY5-7 (ATCC Accession No. CRL-11995). In another embodiment, the mammalian cell is a mouse

embryonic NIH-3T3 cell containing the plasmid pcEXV-hY5 and designated N-hY5-8 (ATCC Accession No. CRL-11994). These cells were deposited on November 15, 1995 with the American Type Culture Collection (ATCC) 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, and were accorded ATCC Accession Nos. CRL-11995 and CRL-11994, respectively.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid molecule encoding a Y5 receptor. In an embodiment, the nucleic acid is DNA.

This nucleic acid produced can either be DNA or RNA. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs.

This nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid encoding the human Y5 receptors can be used as a probe. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule which encodes the Y5 receptor into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and

harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

RNA probes may be generated by inserting the DNA which encodes the Y5 receptor downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

This invention also provides a nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a sequence of a nucleic acid which is complementary to the mammalian nucleic acid encoding a Y5 receptor. This nucleic acid may either be a DNA or RNA molecule. This invention further provides a nucleic acid probe molecule of at least 15 nucleotides which is complementary to a unique fragment of the sequence of the nucleic acid molecule encoding a Y5 receptor. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides which is complementary to the antisense sequence of a unique fragment of the sequence of a nucleic acid molecule encoding a Y5 receptor. In one embodiment, the Y5 receptor is a mammalian receptor. In further embodiments, the Y5 receptor is a human, rat, or canine receptor.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a Y5 receptor so as to prevent translation of the mRNA.

This invention provides an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of a Y5 receptor.

This invention provides an antisense oligonucleotide of a Y5 receptor comprising chemical analogues of nucleotides.

This invention further provides an antibody directed to a Y5 receptor. This invention also provides an antibody directed to a human Y5 receptor.

This invention also provides a monoclonal antibody directed to an epitope of a human Y5 receptor present on the surface of a Y5 receptor expressing cell.

Additionally, this invention provides a pharmaceutical composition comprising an amount of the oligonucleotide effective to reduce activity of a human Y5 receptor by passing through a cell membrane and binding specifically with mRNA encoding a human Y5 receptor in the cell so as to prevent its translation and a pharmaceutically acceptable carrier capable of passing through a cell membrane. In an embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA. In another embodiment, the substance which inactivates mRNA is a ribozyme.

This invention further provides the above-described pharmaceutical composition, wherein the pharmaceutically acceptable carrier capable of passing through a cell membrane comprises a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type.

This invention additionally provides a pharmaceutical composition comprising an amount of an antagonist effective to reduce the activity of a human Y5 receptor and a pharmaceutically acceptable carrier. This invention also provides a pharmaceutical composition comprising an amount of an agonist effective to increase

activity of a Y5 receptor and a pharmaceutically acceptable carrier. This invention further provides a pharmaceutical composition comprising and effective amount of a chemical compound identified by the above-described methods and a pharmaceutically acceptable carrier. This invention also provides the above-described pharmaceutical composition which comprises an amount of the antibody effective to block binding of a ligand to the Y5 receptor and a pharmaceutically acceptable carrier.

As used herein, "pharmaceutically acceptable carriers" means any of the standard pharmaceutically acceptable carriers. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water and emulsions, such as oil/water emulsions.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a human Y5 receptor.

This invention provides a transgenic nonhuman mammal comprising a homologous recombination knockout of the native Y5 receptor.

This invention provides a transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a human Y5 receptor so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a Y5 receptor and which hybridizes to mRNA encoding a Y5 receptor thereby reducing its translation.

This invention provides the above-described transgenic nonhuman mammal, wherein the DNA encoding a human Y5 receptor additionally comprises an inducible promoter.

This invention provides the transgenic nonhuman mammal, wherein the DNA encoding a human Y5 receptor additionally

comprises tissue specific regulatory elements.

In an embodiment, the transgenic nonhuman mammal is a mouse.

Animal model systems which elucidate the physiological and behavioral roles of Y5 receptor are produced by creating transgenic animals in which the activity of the Y5 receptor is either increased or decreased, or the amino acid sequence of the expressed Y5 receptor is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a Y5 receptor, by microinjection, electroporation, retroviral transfection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal or 2) Homologous recombination of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these Y5 receptor sequences. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native Y5 receptors but does express, for example, an inserted mutant Y5 receptor, which has replaced the native Y5 receptor in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added Y5 receptors, resulting in overexpression of the Y5 receptors.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in

an appropriate medium such as M2 medium. DNA or cDNA encoding a Y5 receptor is purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

This invention also provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises contacting a cell transfected with and expressing DNA encoding the Y5 receptor, or a membrane fraction prepared from a cell extract of such cells, with the ligand under conditions permitting binding of ligands to such receptor, detecting the presence of any such ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand specifically binds to the Y5 receptor.

This invention provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises contacting a cell transfected with and expressing DNA encoding the Y5 receptor, or a membrane

fraction from a cell extract of such cells, with the ligand under conditions permitting binding of ligands to such receptor, detecting the presence of any such ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand specifically binds to the Y5 receptor, wherein the Y5 receptor has substantially the same amino acid sequence shown in Figure 6.

This invention provides a method for determining whether a ligand can specifically bind to a Y5 receptor which comprises contacting a cell transfected with and expressing DNA encoding the Y5 receptor, or a membrane fraction of a cell extract of such cells, with the ligand under conditions permitting binding of ligands to such receptor, detecting the presence of any such ligand specifically bound to the Y5 receptor, and thereby determining whether the ligand specifically binds to the Y5 receptor, wherein the Y5 receptor is characterized by an amino acid sequence in the transmembrane region having 60% homology or higher to the amino acid sequence in the transmembrane region of the Y5 receptor shown in Figure 6.

In one embodiment of the above methods, the Y5 receptor is a human Y5 receptor. In another embodiment of the above methods, the Y5 receptor is a rat Y5 receptor. In still another embodiment of the above methods, the Y5 receptor is a canine Y5 receptor.

This invention provides a method for determining whether a ligand is a Y5 receptor agonist which comprises contacting a cell transfected with and expressing a Y5 receptor, or a membrane frction from a cell extract of such cells, with the ligand under conditions permitting activation of a functional Y5 receptor response, detecting a functional increase in Y5 receptor activity, and thereby determining whether the ligand is a Y5

receptor agonist. This invention further provides a method for determining whether a ligand is a Y5 receptor agonist which comprises contacting a cell transfected with and expressing a Y5 receptor, or a membrane fraction prepared from a cell extract of such cells, with the ligand under conditions permitting activation of the Y5 receptor, detecting an increase in Y5 receptor activity, and thereby determining whether the ligand is a Y5 receptor agonist.

In one embodiment of the above-described methods, the Y5 receptor is a human Y5 receptor. In another embodiment, the Y5 receptor is a rat Y5 receptor. In a further embodiment, the Y5 receptor is a canine Y5 receptor.

This invention also provides a method for determining whether a ligand is a Y5 receptor antagonist which comprises contacting a cell transfected with and expressing nucleic acid encoding a Y5 receptor, or a membrane fraction from a cell extract of such cells, with the ligand in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of a functional Y5 receptor response, detecting a decrease in Y5 receptor activity, and thereby determining whether the ligand is a Y5 receptor antagonist. This invention further provides a method for determining whether a ligand is a Y5 receptor antagonist which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor, or a membrane fraction from a cell extract of such cells, with the ligand in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of the Y5 receptor, detecting a decrease in Y5 receptor activity, and thereby determining whether the ligand is a Y5 receptor antagonist.

In one embodiment of the above-described methods, the Y5

receptor is a human Y5 receptor. In another embodiment, the Y5 receptor is a rat Y5 receptor. In a further embodiment, the Y5 receptor is a canine Y5 receptor. In an embodiment of the methods described hereinabove and hereinbelow, the cell is a *Xenopus* cell such as an oocyte or melanophore cell. In another embodiment of the methods described herein, the cell is a neuronal cell such as the glial cell line C6. In yet another embodiment of the methods described herein, the cell is non-neuronal in origin. In a further embodiment, the non-neuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell. In still further embodiments of the methods described herein, the cell may be an insect cell such as a Sf-9 cell or Sf-21 cell. In one embodiment of the above-described methods, the ligand is not previously known.

This invention additionally provides a Y5 receptor agonist detected by the above-described method. This invention also provides a Y5 receptor antagonist detected by the above-described method.

This invention provides a method of screening a plurality of chemical compounds not known to bind to a Y5 receptor to identify a compound which specifically binds to the Y5 receptor which comprises (a) contacting a cell transfected with and expressing DNA encoding the Y5 receptor, or a membrane fraction from a cell extract of such cells, with a compound known to bind specifically to the Y5 receptor; (b) contacting the preparation of step (a) with the plurality of compounds not known to bind specifically to the Y5 receptor, under conditions permitting binding of compounds known to bind to the Y5 receptor; (c) determining whether the binding of the compound known to bind to the Y5 receptor is reduced in the presence of the compounds, relative to the binding of the compound in the absence of the plurality of

compounds; and if so (d) separately determining the binding to the Y5 receptor of each compound included in the plurality of compounds, so as to thereby identify the compound which specifically binds to the Y5 receptor.

Such competitive binding assays provide an efficient means to assess the receptor binding of chemical compounds either singly or in mixtures such as may be present in extracts of natural products or generated using combinatorial chemical synthetic methods for the production of peptidyl and non-peptidyl compounds.

This invention provides a method of screening a plurality of chemical compounds not known to activate a Y5 receptor to identify a compound which activates the Y5 receptor which comprises (a) contacting a cell transfected with and expressing the Y5 receptor, or with a membrane fraction from a cell extract of such cells, with the plurality of compounds not known to bind specifically to the Y5 receptor, under conditions permitting activation of the Y5 receptor; (b) determining whether the activity of the Y5 receptor is increased in the presence of the compounds; and if so (c) separately determining whether the activation of the Y5 receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound which activates the Y5 receptor.

This invention further provides a method of screening a plurality of chemical compounds not known to inhibit the activation of a Y5 receptor to identify a compound which inhibits the activation of the Y5 receptor, which comprises (a) contacting a cell transfected with and expressing the Y5 receptor, or a membrane fraction from a cell exttact of such cells, with the plurality of compounds in the presence of a known Y5 receptor agonist, under conditions permitting activation of the Y5

receptor; (b) determining whether the activation of the Y5 receptor is reduced in the presence of the plurality of compounds, relative to the activation of the Y5 receptor in the absence of the plurality of compounds; and if so (c) separately determining the inhibition of activation of the Y5 receptor for each compound included in the plurality of compounds, so as to thereby identify the compound which inhibits the activation of the Y5 receptor.

In one embodiment of the above-described methods the Y5 receptor is a human Y5 receptor. In another embodiment, the Y5 receptor is a rat Y5 receptor. In a further embodiment, the Y5 receptor is a canine Y5 receptor. In an embodiment of the methods described herein, the cell is a *Xenopus* cell such as an oocyte or melanophore cell. In another embodiment, the cell is a mammalian cell. In a further embodiment, the mammalian cell is non-neuronal in origin. The cell may be a COS-7 cell, CHO cell, a 293 human embryonic kidney cell, a LM(tk-) cell, or an NIH-3T3 cell. In still further embodiments, the cell is an insect cell such as a Sf-9 cell, Sf-21 cell, or HighFive cell.

Additionally, this invention provides a method of screening drugs to identify drugs which specifically bind to a Y5 receptor on the surface of a cell which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor, or a membrane fraction from a cell extract of such cells, with a plurality of drugs under conditions permitting binding of drugs to the Y5 receptor, determining those drugs which specifically bind to the transfected cell, and thereby identifying drugs which specifically bind to the Y5 receptor.

This invention provides a method of screening drugs to identify drugs which act as agonists of a Y5 receptor

which comprises contacting a cell transfected with and expressing DNA encoding a Y5 receptor with a plurality of drugs under conditions permitting the activation of a functional Y5 receptor response, determining those drugs which activate such receptor in the cell, and thereby identify drugs which act as Y5 receptor agonists.

This invention provides a method of screening drugs to identify drugs which act as Y5 receptor antagonists which comprises contacting cells transfected with and expressing DNA encoding a Y5 receptor, or a membrane fraction from a cell extract of such cells, with a plurality of drugs in the presence of a known Y5 receptor agonist, such as PYY or NPY, under conditions permitting the activation of a functional Y5 receptor response, determining those drugs which inhibit the activation of the receptor in the mammalian cell, and thereby identifying drugs which act as Y5 receptor antagonists. In one embodiment of the above-described methods, the cell is a mammalian cell. In another embodiment, the cell is nonneuronal in origin.

This invention also provides a process for identifying a chemical compound which specifically binds to a Y5 receptor, which comprises contacting nonneuronal cells expressing on their cell surface the Y5 receptor, or a membrane fraction from a cell extract of such cells, with the chemical compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the Y5 receptor.

This invention further provides a process involving competitive binding for identifying a chemical compound which specifically binds to a Y5 receptor which comprises separately contacting nonneuronal cells expressing on their cell surface a Y5 receptor, or a membrane fraction from a cell extract of such cells, with both the chemical

compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the Y5 receptor, a decrease in the binding of the second chemical compound to the Y5 receptor in the presence of the chemical compound indicating that the chemical compound binds to the Y5 receptor.

This invention additionally provides a process for determining whether a chemcial compound specifically binds to and activates a Y5 receptor, which comprises contacting nonneuronal cells producing o second messenger response and expressing on their cell surface a Y5 receptor, or a membrane fraction from a cell extract of such cells, with the chemical compound under conditions suitable for activation of the Y5 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in second messenger response in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor.

This invention also provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a Y5 receptor, or a membrane fraction from a cell extract of such cells, with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the chemical compound and the second chemical compound, a smaller change in second messenger

response in the presence of both the chemical compound and the second chemical compound indicating that the chemical compound inhibits activation of the Y5 receptor.

In one embodiment of the above-described methods, the second messenger comprises adenylate cyclase activity and the change in second messenger response is a decrease in adenylate cyclase activity. In a further embodiment, the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a smaller decrease in the level of adenylate cyclase activity in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the second messenger comprises intracellular calcium levels and the change in second messenger response is an increase in intracellular calcium levels. In a further embodiment, the second messenger comprises intracellular calcium levels and the change in second messenger response is a smaller increase in the level of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

In an embodiment of any of the above-described methods, the cell is a mammalian cell. In a further embodiment, the cell is a COS-7 cell, a 293 human embryonic kidney cell, an LM(tk-) cell or an NIH-3T3 cell. It is further to be understood that any of the cells described herein, or any other appropriate host cell, may be used to express the Y5 receptors of the subject invention in any of the above-described embodiments. In one embodiment, the Y5 receptor is a human Y5 receptor. In further embodiments, the Y5 receptor is a rat or a canine Y5 receptor.

The binding and functional assays described herein may be

performed using any cells which express the Y5 receptors of the subject invention, including, but not limited to, cells transfected with exogenous nucleic acid encoding Y5 receptors, as well as cultured cells or cell lines cultured under conditions which lead to expression of Y5 receptors detectable by either binding or functional assays.

This invention also provides for any of the above methods for determining whether a compound activates or inhibits activation of any of the Y5 receptors described herein, wherein the activation is determined not by means of a second messenger response, but by effects of receptor activation which may occur prior to or independent of a second messenger response. In an embodiment, measurement of the second messenger response is replaced with measurement of a change in the binding of GTPγS (a non-hydrolyzable analog of GTP) to cells transfected with and expressing a Y5 receptor or to a membrane fraction from such cells. Preferably, the cells are nonneuronal cells. In a further embodiment, an increase in GTPγS binding to the cells or the membrane fraction in the presence of a compound indicates that the compound activates the Y5 receptor. In yet another embodiemnt, a smaller increase in GTPγS binding to the cells or membrane fraction in the presence of both a compound known to activate the receptor and a test compound, relative to the increase in GTPγS binding in the presence of only the compound known to activate the receptor, indicates that the test compound inhibits activation of the Y5 receptor. In other embodiemnts, activation or inhibition of activation of any of the Y5 receptors disclosed herein may be measured by other means not requiring a second messneger, such as activation of MAP kinase, or activation of a reporter gene system, or by activation of immediate early genes, which are well known in the art.

This invention provides a process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting nonneuronal cells expressing a Y5 receptor, or a membrane fraction from a cell extract of such cells, with the chemical compound under conditions suitble for activation of the Y5 receptor, and measuring the binding of GTPγS to the cells or membrane fraction, in the presence and in the absence of the chemical compound, a change in the binding of GTPγS in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor. This invention further provides a process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting nonneuronal cells expressing a Y5 receptor, with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor, and measuring binding of GTPγS to the cell or membrane fraction in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in GTPγS binding in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of a Y5 receptor. In one embodiment of the above-described methods the change in binding is an increase in GTPγS binding. In another embodiment, the change in binding is a smaller increase in GTPγS binding in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound. In another embodiment, the cells are not intact.

It is known in the art that that in cell lines, the

expression level of endogenous receptors can be increased several-fold by treatment with compounds such as Il-1ß (Menke, et al., 1994), NGF (Dimaggio, et al., 1994) or glucocorticoids (Larsen, et al., 1994). Such treatment may allow screening of compounds at Y5 receptors in cell lines expressing previously undetectable levels of endogenous Y5 receptors, without transfecting such cell lines with the Y5 receptor. One may also create recombinant cell lines, whereby the normal promoter may be replaced with promoter element(s) that allow increased expression of the Y5 gene, thereby allowing one to screen compounds using the recombinant cell line. Such cells and cell lines may be used with any of the above-described methods or processes.

This invention provides a pharmaceutical composition comprising a drug identified by the above-described methods and a pharmaceutically acceptable carrier.

This invention provides a method of detecting expression of Y5 receptor by detecting the presence of mRNA coding for the Y5 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the above-described nucleic acid probe under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the Y5 receptor by the cell.

This invention provides a method of treating obesity and other disorders associated with excess eating (e.g., bulimia) in which a Y5 receptor antagonist is administered in combination with existing therapies. An example os such a drug is dexfenfluramine, a serotonin uptake inhibitor (McTavish, D. and R.C. Heel, Drugs 43(5):713-733 (1992)). Administration of dexfenfluramine results in significant weight loss after about one month of therapy, with maximal weight loss occurring in the

first six months of therapy. It is noteworthy that after discontinuation of drug therapy an increas in body weight is observed after about two months. One study reports that no statistically significant differences from placebo were observable by five months after discontinuing drug therapy (O'Connor, H.T. et al., Int. J. Obes. Relat. Metab. Disord. 19(3):30-337 (1991)). Although the ptotential usefulness of sibutramine therapy has not been fully explored, combinations of sibutramine and a Y5 receptor antagonist may also prove useful.

This invention provides a method of decreasing feeding behavior in a subject which comprises administering to the subject a compound which is a Y5 receptor antagonist and a compound which is monoamine neurotransmitter uptake inhibitor, wherein the amount of the Y5 antagonist and the monoamine neurotransmitter uptake inhibitor are effective to decrease the feeding behavior of the subject. This invention also provides the use of a compound which is a Y5 receptor antagonist and a compound which is a monoamine neurotransmitter uptake inhibitor for the preparation of a pharmaceutical composition for decreasing feeding behavior in a subject, wherein the amount of the Y5 receptor antagonist and the amount of the monoamine neurotransmitter uptake inhibitor is effective to decrease feeding behavior in the subject. In one embodiment of the above-described methods, the Y5 receptor antagonist and the monoamine neurotransmitter uptake inhibitor aer administered in combination. In another embodiment, the Y5 receptor antagonist and the monoamine neurotransmitter uptake inhibitor are administered once. In a further embodiment, the Y5 receptor antagonist and the monoamine neurotransmitter uptake inhibitor are administered separately. In another embodiment, the Y5 receptor antagonist and the monoamine neurotransmitter uptake inhibitor are administered once. In one embodiment, the Y5 receptor antagonist is

administered for about two weeks to about six months. In another embodiment, the monoamine neurotransmitter uptake inhibitor is administered for about one month to about six months. In a further embodiment, the Y5 receptor antagonist and the monoamine neurotransmitter uptake inhibitor are administered repeatedly. In another embodiment, the Y5 receptor antagonist is administered for about two weeks to about six months. In one embodiment, the monoamine neurotransmitter uptake inhibitor is administered for about one month to about six months. In another embodiment, the neurotransmitter uptake inhibitor is administered for about one month to about three months. In separate emodiments, the monoamine neurotransmitter uptake inhibitor may be fenfluramine, dexfenfluramine, or sibutramine. In another embodiment, the compound is administered in a pharmaceutical composition comprising a sustained release formula.

This invention provides a method of decreasing feeding behavior of a subject which comprises administering to the subject a compound which is a galanin receptor antagonist and a compound which is a Y5 receptor antagonist, wherein the amount of the antagonists is effective to decrease feeding behavior of the subject. In one embodidment, the galanin receptor antagonist and the Y5 receptor antagonist are administered in combination. In another embodiment the galanin receptor antagonist and the Y5 receptor antagonist are administered once. In a further embodiment the galanin receptor antagonist and the Y5 receptor antagonist are administered separately. In another embodiment the galanin receptor antagonist and the Y5 receptor antagonist are administered once. In an embodiment the galanin receptor antagonist is administered for about 1 week to about 2 weeks. In a further embodiment the Y5 receptor antagonist is administered for about 1 week to

about 2 weeks. In another embodiment, the galanin receptor antagonist and the Y5 receptor antagonist are administered repeatedly. In an embodiment, the galanin receptor antagonist is administered for about 1 week to about 2 weeks. In separate embodiments, the galanin receptor is a GALR2 receptor or a GALR3 receptor. In another embodiment the compound is administered in a pharmaceutical composition comprising a sustained release formulation.

This invention provides a method of treating an abnormality in a subject, wherein the abnormality is alleviated by the inhibition of a Y5 receptor which comprises administering to a subject an amount of the above-described pharmaceutical composition effective to decrease the activity of the Y5 receptor in the subject and thereby treat the abnormality.

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a Y5 receptor which comprises administering to a subject an amount of the above-described pharmaceutical composition effective to incresase the activation of the Y5 receptor in the subject and thereby treate the abnormality.

This invention provides a method of treating an abnormality in a subject, wherein the abnormality is alleviated by the decreasing the activity of a Y5 receptor which comprises administering to a subject an amount of the above-described pharmaceutical composition effective to decrease the activity of the Y5 receptor and thereby treat the abnormality.

In one embodiment of the above-described methods, the abnormality is obesity. In another embodiment, the abnormality is bulimia.

This invention provides a method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a Y5 receptor which comprises administering to a subject an effective amount of a Y5 receptor agonist. In a further embodiment, the abnormal condition is anorexia. In a separate embodiment, the abnormal condition is a sexual/reproductive disorder. In another embodiment, the abnormal condition is depression. In another embodiment, the abnormal condition is anxiety.

In an embodiment, the abnormal condition is gastric ulcer. In a further embodiment, the abnormal condition is memory loss. In a further embodiment, the abnormal condition is migraine. In a further embodiment, the abnormal condition is pain. In a further embodiment, the abnormal condition is epileptic seizure. In a further embodiment, the abnormal condition is hypertension. In a further embodiment, the abnormal condition is cerebral hemorrhage. In a further embodiment, the abnormal condition is shock. In a further embodiment, the abnormal condition is congestive heart failure. In a further embodiment, the abnormal condition is sleep disturbance. In a further embodiment, the abnormal condition is nasal congestion. In a further embodiment, the abnormal condition is diarrhea.

This invention further provides a method of treating obesity in a subject which comprises administering to the subject an effective amount of a Y5 receptor antagonist. This invention also provides a method of treating anorexia in a subject which comprises administering to the subject an effective amount of a Y5 receptor agonist.

In addition, this invention provides a method of treating bulimia nervosa in a subject which comprises administering to the subject an effective amount of a Y5

receptor antagonist.

This invention provides a method of inducing a subject to eat which comprises administering to the subject an effective amount of a Y5 receptor agonist. In one embodiment, the subject is a vertebrate. In another embodiment, the subject is a human. In another embodiment, the subject is a rat. In another embodiment, the subject is a canine subject. This invention also provides a method of increasing the consumption of a food product by a subject which comprises administering to the subject a composition of the food product and an amount of a Y5 receptor agonist. In one embodiment, the subject is a vertebrate. In another embodiment, the subject is a human, a rat or a canine subject.

This invention also provides a method of treating abnormalities which are alleviated by reduction of activity of a human Y5 receptor which comprises administering to a subject an amount of the above-described pharmaceutical composition effective to reduce the activity of human Y5 receptor and thereby alleviate abnormalities resulting from overactivity of a human Y5 receptor. This invention further provides a method of treating an abnormal condition related to an excess of Y5 receptor activity which comprises administering to a subject an amount of the pharmaceutical composition effective to block binding of a ligand to the Y5 receptor and thereby alleviate the abnormal condition.

This invention additionally provides a method of detecting the presence of a Y5 receptor on the surface of a cell which comprises contacting the cell with the antibody capable of binding to the Y5 receptor under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a Y5

receptor on the surface of the cell.

This invention also provides a method of determining the physiological effects of varying levels of activity of a Y5 receptor which comprises producing a transgenic nonhuman mammal whose levels of Y5 receptor activity are varied by use of an inducible promoter which regulates Y5 receptor expression. This invention further provides a method of determining the physiological effects of varying levels of activity of a Y5 receptors which comprises producing a panel of transgenic nonhuman mammals each expressing a different amount of Y5 receptor.

This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from overactivity of a Y5 receptor comprising administering a substance to the above-described transgenic nonhuman mammals, and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overactivity of a Y5 receptor.

This invention also provides a method for treating abnormalities resulting from overactivity of a Y5 receptor which comprises administering to a subject an amount of the above-described pharmaceutical composition effective reduce the activation of the Y5 receptor and thereby alleviate the abnormalities resulting from overactivity of a Y5 receptor.

This invention further provides a method for identifying a substance capable of alleviating the abnormalities resulting from underactivity of a Y5 receptor comprising administering the substance to the above-described transgenic nonhuman mammals and determining whether the substance alleviates the physical and behavioral

abnormalities displayed by the transgenic nonhuman mammal as a result of underactivity of a Y5 receptor.

This invention additionally provides a method for treating the abnormalities resulting from underactivity of a Y5 receptor which comprises administering to a subject an amount of the above-described pharmaceutical composition effective to increase the activation of the Y5 receptor and thereby alleviate the abnormalities resulting from underactivity of a Y5 receptor.

This invention provides a method for diagnosing a predisposition to a disorder associated with the activity of a specific allelic form of a Y5 receptor which comprises: a. obtaining DNA from the subject to be tested; digesting the DNA with restriction enzymes; c. separating the resulting DNA fragments; d. contacting the fragments with a detectably labeled nucleic acid probe capable of specifically hybridizing with a sequence uniquely present within the sequence of a nucleic acid encoding the allelic form of the Y5 receptor; and e. detecting the presence of labeled probe hybridized to the DNA fragments from the subject being tested, the presence of such hybridized probe indicating that the subject is predisposed to the disorder.

This invention also provides a method of preparing an isolated Y5 receptor which comprises: a. inducing cells to express the Y5 receptor; b. recovering the receptor from the resulting cells; and c. purifying the receptor so recovered. This invention further provides a method of preparing the isolated Y5 receptor which comprises: a. inserting nucleic acid encoding Y5 receptor in a suitable vector adapted for expression in a bacterial, yeast, insect, or mammalian cell operatively linked to the nucleic acid encoding the Y5 receptor as to permit expression thereof; b. inserting the resulting vector in

a suitable host cell so as to obtain a cell which produces the Y5 receptor; c. recovering the receptor produced by the resulting cell; and d. purifying the receptor so recovered.

This invention provides a method for detecting in a subject the presence of a restriction fragment length polymorphism associated with a genomic locus which encompasses both a Y1 and a Y5 receptor gene which comprises: a) obtaining a sample of DNA from the subject; b) digesting the DNA with a restriction enzyme; c) separating the resulting DNA fragments; d) contacting the DNA fragments with a detectably labeled nucleic acid probe which specifically hybridizes with a sequence uniquely present within the sequence associated with the polymorphism; and e) detecting whether the probe hybridizes to the DNA fragments, the presence of the labeled probe hybridized to the DNA fragment indicating the presence of the restriction fragment length polymorphism.

In an embodiment of the above-described method, the restriction enzyme is PstI. In another embodiment, the subject is a human. In still another embodiment, the PstI polymorphism is associated with susceptibility to modification of feeding behavior using a Y5-selective compound. In various embodiments, the feeding behavior is anorexia or bulimia, or the feeding behavior is associated with obesity.

In an embodiment of any of the above-described methods, the subject is a human. In another embodiment, the subject is a non-human animal. In still another embodiment, the subject is a mammal. In yet another embodiment, the subject is a bovine, equine, canine or feline.

This invention provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the binding of the compound to the human Y5 receptor is characterized by a K_i less than 100 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration, and wherein the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor.

In an embodiment of the above-described method, the binding of the compound to each of the human Y1, human Y2, and human Y4 receptors is characterized by a K_i greater than 500 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In another embodiment, the binding of the compound to each of the human Y1, human Y2, and human Y4 receptors is characterized by a K_i greater than 1000 nanomolar.

This invention also provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the binding of the compound to the human Y5 receptor is characterized by a K_i less than 5 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration.

In an embodiment of the above-described method, the compound to each of the human Y1, human Y2, and human Y4 receptors is characterized by a K_i greater than 5 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In another embodiment of

the above-described method, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor. In yet another embodiment, the binding of the compound to each of the human Y1, human Y2 and human Y4 receptors is characterized by a K_i greater than 50 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration. In still another embodiment, the binding of the compound to each of the human Y1, human Y2 and human Y4 receptors is characterized by a K_i greater than 100 nanomolar.

This invention further provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor. In an embodiment of the above-described method, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor, and greater than 26-fold higher than the affinity with which the compound binds to the human Y1 receptor. In another embodiment of the above-described methods, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor, and greater than 22-fold higher than the affinity with which the compound binds to the human Y2 receptor. In still another embodiment of the above-described methods, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type

receptor, and greater than 34-fold higher than the affinity with which the compound binds to the human Y4 receptor.

In another embodiment of the above-described methods, the compound binds to the human Y5 receptor with an affinity a) greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor; b) greater than 22-fold higher than the affinity with which the compound binds to the human Y2 receptor; and c) greater than 34-fold higher than the affinity with which the compound binds to the human Y4 receptor. In another embodiment of the above-described methods, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor, and with an affinity a) greater than 26-fold higher than the affinity with which the compound binds to the human Y1 receptor; b) greater than 22-fold higher than the affinity with which the compound binds to the human Y2 receptor; and c) and greater than 34-fold higher than the affinity with which the compound binds to the human Y4 receptor. In yet another embodiment of the above described methods, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor, and greater than 100-fold higher than the affinity with which the compound binds to the human Y1 receptor. In a further embodiment of the above described methods, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor, and greater than 165-fold higher than the affinity with which the compound binds to the human Y2 receptor.

In another embodiment of the above described methods, the

compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor, and greater than 143-fold higher than the affinity with which the compound binds to the human Y4 receptor. In yet another embodiment of the above described methods, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor and a) greater than 143-fold higher than the affinity with which the compound binds to the human Y4 receptor; and b) greater than 165-fold higher than the affinity with which the compound binds to the human Y2 receptor. In still yet another embodiment of the above described methods, the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor, and a) greater than 143-fold higher than the affinity with which the compound binds to the human Y4 receptor; b) greater than 165-fold higher than the affinity with which the compound binds to the human Y2 receptor; and c) greater than 100-fold higher than the affinity with which the compound binds to the human Y1 receptor.

This invention additionally provides a method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the compound binds to the human Y5 receptor with an affinity greater than 500-fold higher than the affinity with which the compound binds to each of the human Y1, human Y2, and human Y4 receptors.

This invention also provides a method of treating a subject's feeding disorder which comprises administering

to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the compound binds to the human Y5 receptor with an affinity greater than 1400-fold higher than the affinity with which the compound binds to each of the human Y1, human Y2, and human Y4 receptors.

In an embodiment of any of the above methods, the feeding disorder is obesity or bulimia. In a further embodiment of any of the above methods, the subject is a vertebrate, a mammal, a human or a canine.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

MATERIALS AND METHODS

cDNA Cloning

Total RNA was prepared by a modification of the guanidine thiocyanate method (Kingston, 1987), from 5 grams of rat hypothalamus (Rockland, Gilbertsville, PA). Poly A^+RNA was purified with a FastTrack kit (Invitrogen Corp., San Diego, CA). Double stranded (ds) cDNA was synthesized from 7 μg of poly A^+ RNA according to Gubler and Hoffman (Gubler and Hoffman, 1983), except that ligase was omitted in the second strand cDNA synthesis. The resulting ds-cDNA was ligated to BstXI/EcoRI adaptors (Invitrogen Corp.), the excess of adaptors was removed by chromatography on Sephacryl 500 HR (Pharmacia®-LKB) and the ds-cDNA size selected on a Gen-Pak Fax HPLC column (Millipore Corp., Milford, MA). High molecular weight fractions were ligated in pEXJ.BS (A cDNA cloning expression vector derived from pcEXV-3; Okayama and Berg, 1983; Miller and Germain, 1986) cut by BstXI as described by Aruffo and Seed (Aruffo and Seed, 1987). The ligated DNA was electroporated in E.coli MC 1061 F^+ (Gene Pulser, Biorad). A total of 3.4×10^6 independent clones with an insert mean size of 2.7 kb could be generated. The library was plated on Petri dishes (Ampicillin selection) in pools of 6.9 to 8.2×10^3 independent clones. After 18 hours amplification, the bacteria from each pool were scraped, resuspended in 4 mL of LB media and 1.5 mL processed for plasmid purification with a QIAprep-8 plasmid kit (Qiagen Inc, Chatsworth, CA). 1 ml aliquots of each bacterial pool were stored at -85°C in 20% glycerol.

Isolation of a cDNA clone encoding an atypical rat hypothalamic NPY5 receptor

DNA from pools of ≈ 7500 independent clones was transfected into COS-7 cells by a modification of the DEAE-dextran procedure (Warden and Thorne, 1968). COS-7

cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin, 2mM L-glutamine (DMEM-C) at 37°C in 5% CO_2. The cells were seeded one day before transfection at a density of 30,000 cells/cm^2 on Lab-Tek chamber slides (1 chamber, Permanox slide from Nunc Inc., Naperville, IL). On the next day, cells were washed twice with PBS, 735 μl of transfection cocktail was added containing 1/10 of the DNA from each pool and DEAE-dextran (500 μg/ml) in Opti-MEM I serum free media (Gibco BRL LifeTechnologies Inc. Grand Island, NY). After a 30 min. incubation at 37°C, 3 ml of chloroquine (80 μM in DMEM-C) was added and the cells incubated a further 2.5 hours at 37°C. The media was aspirated from each chamber and 2 ml of 10% DMSO in DMEM-C added. After 2.5 minutes incubation at room temperature, the media was aspirated, each chamber washed once with 2 ml PBS, the cells incubated 48 hours in DMEM-C and the binding assay was performed on the slides. After one wash with PBS, positive pools were identified by incubating the cells with 1 nM ($3x10^6$ cpm per slide) of porcine [^{125}I]-PYY (NEN; SA=2200Ci/mmole) in 20 mM Hepes-NaOH pH 7.4, $CaCl_2$ 1.26 mM, $MgSO_4$ 0.81 mM, KH_2PO_4 0.44 mM, KCL 5.4, NaCl 10mM, 0.1% BSA, 0.1% bacitracin for 1 hour at room temperature. After six washes (three seconds each) in binding buffer without ligand, the monolayers were fixed in 2.5% glutaraldehyde in PBS for five minutes, washed twice for two minutes in PBS, dehydrated in ethanol baths for two minutes each (70, 80, 95, 100%) and air dried. The slides were then dipped in 100% photoemulsion (Kodak type NTB2) at 42°C and exposed in the dark for 48 hours at 4°C in light proof boxes containing drierite. Slides were developed for three minutes in Kodak D19 developer (32 g/L of water), rinsed in water, fixed in Kodak fixer for 5 minutes, rinsed in water, air dried and mounted with Aqua-Mount (Lerner Laboratories, Pittsburgh, PA). Slides were screened at 25x total magnification. A single clone,

CG-18, was isolated by SIB selection as described (Mc Cormick, 1987). DS-DNA was sequenced with a Sequenase kit (US Biochemical, Cleveland, OH) according to the manufacturer. Nucleotide and peptide sequence analysis were performed with GCG programs (Genetics Computer Group, Madison, WI).

Isolation of the human Y5 homolog

Using rat oligonucleotide primers in TM 3 (sense primer; position 484-509 in fig. 1A) and in TM 6 (antisense primer; position 1219-1243 in fig. 3A), applicants screened a human hippocampal cDNA library using the polymerase chain reaction. 1 μl (4 x 10^6 bacteria) of each of 450 amplified pools containing each ≈5000 independent clones and representing a total of 2.2 x 10^6 was subjected directly to 40 cycles of PCR and the resulting products analyzed by agarose gel electrophoresis. One of three positive pools was analyzed further and by sib selection a single cDNA clone was isolated and characterized. This cDNA turned out to be full length and in the correct orientation for expression. DS-DNA was sequenced with a sequenase kit (US Biochemical, Cleveland, OH) according to the manufacturer.

Isolation of the canine Y5 homolog

An alignment of the coding nucleotide sequences of the rat and human Y5 receptors was used to synthesize a pair of PCR primers. A region upstream of TM III which is 100% conserved between rat and human was chosen to synthesize the forward primer CH 156:

5'-TGGATCAGTGGATGTTTGGCAAAG-3' (Seq. I.D. No. 7).

A region at the carboxy end of the 5-6 loop, immediately upstream of TM6, which is also 100% conserved between rat and human sequences was chosen to synthesize the reverse

primer CH153:

5'-GTCTGTAGAAAACACTTCGAGATCTCTT-3' (Seq. I.D. No. 8).

The primers CH156-CH153 were used to amplify 10 ng of poly (A+) RNA from rat brain that was reverse transcribed using the SSII reverse transcriptase (GibcoBRL, Gaithersburg, MD). PCR was performed on single-stranded cDNA with Taq Polymerase (Perkin Elmer-Roche Molecular Systems, Branchburg, NJ)under the following conditions: 94°C for 1 min, 60°C for 1 min and 72°C for 1 min for 40 cycles. The resulting 798 bp PCR DNA fragment was subcloned in pCR Script (Stratagene, La Jolla, CA) and sequenced using a sequenase kit (USB, Cleveland, OH) and is designated Y5-bd-5.

<u>3' and 5' RACE</u>

It was attempted to isolate the missing 3' and 5' ends of the beagle dog Y5 receptor sequences by 3' and 5' RACE using a Marathon cDNA amplification kit (Clontech, Palo Alto, CA). From the sequence of the canine (beagle) PCR DNA fragment described above, the following PCR primers were synthesized:

(3' RACE)

CH 204:

5'-CTTCCAGTGTTTCACAGTCTGGTGG-3' (Seq. I.D. No. 9);

CH 218 (nested primer):

5'-CTGAGCAGCAGGTATTTATGTGTTG-3' (Seq. I.D. No. 10);

(5' RACE)

CH 219:

5'-CTGGATGAAGAATGCTGACTTCTTACAG-3' (Seq. I.D. No. 11);

CH 245 (nested primer):

5'-TTCTTGAGTGGTTCTCTTGAGGAGG-3' (Seq. I.D. No. 12).

The 3' and 5' RACE reactions were carried out on canine thalamic cDNA according to the kit specifications, with the primers described above. The resulting PCR DNA products (smear of 0.7 to 10 kb) were purified from an agarose gel and reamplified using the nested primers described above. The resulting discrete DNA bands were again purified from an agarose gel and subcloned in pCR Script (Stratagene, La Jolla, CA).

The nucleotide sequence corresponding to the 3' end of the cDNA was determined and the plasmid designated Y5-bd-8. However, attempts to determine the 5' sequence of the beagle Y5 receptor by 5' RACE were unsuccessful.

As a second approach, a canine brain cDNA library (in the pEXJ vector) was screened by PCR using primers BB33 (TM-3) and BB34 (3-4 loop). Vector-anchored PCR, using primers BB34 and KS938 (pEXJ + strand) or KS939 (pEXJ - strand) was then used to amplify the 5' end from two positive pools. The resulting PCR products (0.6 and 0.57 kb) were purified from an agarose gel and subcloned into the pCR Script vector (Stratagene, La Jolla, CA). The nucleotide sequence of the longer of these products was determined using a sequenase kit (USB, Cleveland, OH) and designated dogY5-16. By comparison to the human Y5 receptor, dogY5-16 lacked the first 18 nucleotides of the Y5 coding sequence.

To obtain the additional 5' sequence, a nitrocellulose membrane (Schleicher and Schuell, Keene, NH) containing 20 μg of HindIII-cut canine genomic DNA (Clontech, Palo Alto, CA) was hybridized with a ^{32}P-labeled oligonucleotide probe (BB53) corresponding to nucleotides 3-35 of dogY5-16. A 4.2 kb hybridizing band was isolated from a replicate agarose gel and subcloned into the pUC18

vector. Vector anchored PCR was performed on one-tenth of the ligation reaction using BB34 (3-4 loop) and BB77 (pUC18 + strand) or BB78 (pUC18 - strand). The resulting PCR products (1.35, 0.87, 0.75 and 0.7 kb) were then re-amplified using BB77 and a nested primer BB70 (nucleotides 94-111 from dogY5-16). The resulting PCR products (0.4, 0.7 and 0.95 kb) were purified from an agarose gel and subcloned in pCR Script (Stratagene, La Jolla, CA). A portion of the 0.95 kb fragment, designated dogY5-2-29, was sequenced using a Sequenase kit (USB, Cleveland, OH).

To obtain a full-length canine Y5 receptor, the primers BB80 (5′ untranslated sequence (UT) from dogY5-2-29) and BB54 (carboxy tail and 3′ UT from Y5-bd-8) were used to amplify 0.36μg of beagle genomic DNA. PCR was performed using Expand High Fidelity polymerase (Boehringer Mannheim Corporation, Indianapolis, IN) under the following conditions: 94°C for 1 min, 63°C for 2 min and 68°C for 3 min for 38 cycles. The resulting 1.4 kb PCR band was purified from an agarose gel and subcloned into pEXJ. Three clones, designated BO10, BO11 and BO12 were sequenced using a sequenase kit (USB, Clevland, OH). The pEXJ derived plasmid comprising clone BO11 was designated cY5-BO11 and was deposited with the ATCC on May 29, 1996, under ATCC Accession No. 97587.

The primers used as described above were as follows:

BB33:
5′- GCCTTTTCTTCAATGTGTGTCAG -3′ (Seq. I.D. No. 15).

BB34:
5′ - CCAGACAGTAGCAATCAGGAAGTAGC -3′ (Seq. I.D. No. 16).

KS938:
5′- AAGCTTCTAGAGATCCCTCGACCTC -3′(Seq. I.D. No. 17).

KS939:
5'- AGGCGCAGAACTGGTAGGTATGGAA -3' (Seq. I.D. No. 18).

BB53:
5'- GAACTCTAAGATGGATTTAGAACTCCAGATTTT -3' (Seq. I.D. No. 19).

BB77:
5'- ATGCTTCCGGCTCGTATGTTGTGTGG -3' (Seq. I.D. No. 20).

BB78:
5'- GCCTCTTCGCTATTACGCCAGCTGGC -3' (Seq. I.D. No. 21).

BB70:
5'- TAGTCATCCCAGACTGGG -3' (Seq. I.D. No. 22).

BB80:
5'- GTAGTCTCCCTCTCAGAATTGATTTATCG -3' (Seq. I.D. No. 23).

BB54:
5'- GGTAAACATGAAGAATTATGACATATGAAGAC -3' (Seq. I.D. No. 24).

Northern Blots

Human brain multiple tissue northern blots (MTN blots II and III, Clontech, Palo Alto, CA) carrying mRNA purified from various human brain areas was hybridized at high stringency according to the manufacturer specifications. The probe was a 0.8 kb DNA PCR fragment corresponding to the TM III - carboxy end of the 5-6 loop in the coding region of the human Y5 receptor subtype.

A rat multiple tissue northern blot (rat MTN blot, Clontech, Palo Alto, CA) carrying mRNA purified from various rat tissues was hybridized at high stringency according to the manufacturer specifications. The probe

was a 0.8 kb DNA PCR fragment corresponding to the TM III - carboxy end of the 5-6 loop in the coding region of the rat Y5 receptor subtype.

Southern Blot

Southern blots (Geno-Blot, clontech, Palo Alto, CA) containing human or rat genomic DNA cut with five different enzymes (8 μg DNA per lane) was hybridized at high stringency according to the manufacturer specifications. The probe was a 0.8 kb DNA PCR fragment corresponding to the TM III - carboxy end of the 5-6 loop in the coding region of the human and rat Y5 receptor subtypes.

Production of Recombinant Baculovirus

A BamHI site directly 5' to the starting methionine of human Y5 was genetically engineered by replacing the beginning ≈100 base pairs of hY5 (i.e. from the starting methionine to an internal EcoRI site) with two overlapping synthetically-derived oligonucleotides (≈100 bases each), containing a 5' BamHI site and a 3' EcoRI site. This permitted the isolation of an ≈1.5 kb Bam HI/Hind III fragment containing the coding region of hY5. This fragment was subcloned into pBlueBacIII™ into the Bam HI/Hind III sites found in the polylinker (construct called pBB/hY5). To generate baculovirus, 0.5 μg of viral DNA (BaculoGold™) and 3 μg of pBB/hY5 were co-transfected into 2 x 10^6 *Spodoptera frugiperda* insect Sf9 cells by calcium phosphate co-precipitation method, as outlined by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual"). The cells were incubated for 5 days at 27°C. The supernatant of the co-transfection plate was collected by centrifugation and the recombinant virus (hY5BB3) was plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks were as described in Pharmingen's manual.

<u>Cell Culture</u>

COS-7 cells were grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 μg/ml streptomycin) at 37°C, 5% CO_2. Stock plates of COS-7 cells were trypsinized and split 1:6 every 3-4 days. Human embryonic kidney 293 cells were grown on 150 mm plates in D-MEM with supplements (minimal essential medium) with Hanks' salts and supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 μg/ml streptomycin) at 37°C, 5% CO_2. Stock plates of 293 cells were trypsinized and split 1:6 every 3-4 days. Mouse fibroblast LM(tk-) cells were grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 μg/mL streptomycin) at 37°C, 5% CO_2. Stock plates of LM(tk-) cells were trypsinized and split 1:10 every 3-4 days.

LM(tk-) cells stably transfected with the human Y5 receptor were routinely converted from an adherent monolayer to a viable suspension. Adherent cells were harvested with trypsin at the point of confluence, resuspended in a minimal volume of complete DMEM for a cell count, and further diluted to a concentration of 10^6 cells/ml in suspension media (10% bovine calf serum, 10% 10X Medium 199 (Gibco), 9 mM $NaHCO_3$, 25 mM glucose, 2 mM L-glutamine, 100 units/ml penicillin/100 μg/ml streptomycin, and 0.05% methyl cellulose). The cell suspension was maintained in a shaking incubator at 37°C, 5% CO_2 for 24 hours. Membranes harvested from cells grown in this manner may be stored as large, uniform batches in liquid nitrogen. Alternatively, cells may be returned to adherent cell culture in complete DMEM by distribution into 96-well microtiter plates coated with poly-D-lysine (0.01 mg/ml) followed by incubation at

37°C, 5% CO_2 for 24 hours. Cells prepared in this manner yielded a robust and reliable NPY-dependent response in cAMP radio-immunoassays as further described hereinbelow.

Mouse embryonic fibroblast NIH-3T3 cells were grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/ml penicillin/100 μg/ml streptomycin) at 37°C, 5% CO_2. Stock plates of NIH-3T3 cells were trypsinized and split 1:15 every 3-4 days.

Sf9 and Sf21 cells were grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27°C, no CO_2. High Five insect cells were grown on 150 mm tissue culture dishes in Ex-Cell 400™ medium supplemented with L-Glutamine, also at 27°C, no CO_2.

Transient Transfection

All receptor subtypes studied (human and rat Y1, human and rat Y2, human and rat Y4, human, rat and canine Y5) were transiently transfected into COS-7 cells by the DEAE-dextran method, using 1 μg of DNA /10^6 cells (Cullen, 1987). The Y1 receptor was prepared using known methods (Larhammar, et al., 1992).

Stable Transfection

Human Y1, human Y2, and rat Y5 receptors were co-transfected with a G-418 resistant gene into the human embryonic kidney 293 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells were selected with G-418. Human Y4 and human Y5 receptors were similarly transfected into mouse fibroblast LM(tk-) cells and NIH-3T3 cells. Canine Y5 receptors also may be similarly transfected into LM(tk-), NIH-3T3 cells or other appropriate host cells. Additional host cells appropriate for transfection of the

Y-type receptors are well known in the art and include, but are not limited to, Chinese hamster ovary cells (CHO), the glial cell line C6, or non-mammalian host cells such as *Xenopus* melanophore cells.

Expression of receptors in *Xenopus* oocytes

Expression of genes in *Xenopus* oocytes is well known in the art (Coleman, Transcription and Translation: A Practical Approach (B.D. Hanes, S.J. Higgins, eds., pp 271-302, IRL Press, Oxford, 1984; Y. Masu, et al. (1987) Nature 329:836-838; Menke, J.G. et al. (1984) J.Biol.Chem. 269(34):21583-21586) and is performed using microinjection into *Xenopus* oocytes of native mRNA or in vitro synthesized mRNA. The preparation of in vitro synthesized mRNA can be performed using various standard techniques (J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Editions, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989) including using T7 polymerase with the mCAP RNA capping kit (Stratagene).

Expression of other G-protein coupled receptors

α_1 Human Adrenergic Receptors: To determine the binding of compounds to human α_1 receptors, LM(tk-) cell lines stably transfected with the genes encoding the α_{1a}, α_{1b}, and α_{1d} receptors were used. The nomenclature describing the α_1 receptors was changed recently, such that the receptor formerly designated α_{1a} is now designated α_{1d}, and the receptor formerly designated α_{1c} is now designated α_{1a} (ref). The cell lines expressing these receptors were deposited with the ATCC before the nomenclature change and reflect the subtype designations formerly assigned to these receptors. Thus, the cell line expressing the receptor described herein as the α_{1a} receptor was deposited with the ATCC on September 25, 1992, under ATCC Accession No. CRL 11140 with the designation L-α_{1C}. The cell line expressing receptor described herein as the α_{1d}

receptor was deposited with the ATCC on September 25, 1992, under ATCC Accession No. CRL 11138 with the designation L-α_{1A}. The cell line expressing the α_{1b} receptor is designated L-α_{1B}, and was deposited on September 25, 1992, under ATCC Accession No. CRL 11139.

α_2 Human Adrenergic Receptors: To determine the binding of compounds to human α_2 receptors, LM(tk-) cell lines stably transfected with the genes encoding the α_{2A}, α_{2B}, and α_{2C} receptors were used. The cell line expressing the α_{2A} receptor is designated L-α_{2A}, and was deposited on November 6, 1992, under ATCC Accession No. CRL 11180. The cell line expressing the α_{2B} receptor is designated L-NGC-α_{2B}, and was deposited on October 25, 1989, under ATCC Accession No. CRL 10275. The cell line expressing the α_{2C} receptor is designated L-α_{2C}, and was deposited on November 6, 1992, under ATCC Accession No. CRL-11181. Cell lysates were prepared as described below (see Radioligand Binding to Membrane Suspensions), and suspended in 25mM glycylglycine buffer (pH 7.6 at room temperature). Equilibrium competition binding assay were performed using [^{3}H]rauwolscine (0.5nM), and nonspecific binding was determined by incubation with 10μM phentolamine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Histamine H_1 Receptor: The coding sequence of the human histamine H_1 receptor, homologous to the bovine H_1 receptor, was obtained from a human hippocampal cDNA library, and was cloned into the eukaryotic expression vector pcEXV-3. The plasmid DNA for the H_1 receptor is designated pcEXV-H1, and was deposited on November 6, 1992, under ATCC Accession No. 75346. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C,

and the supernatant was centrifuged at 30,000 x g for 20 min. at 4°C. The pellet was suspended in 37.8 mM $NaHPO_4$, 12.2 mM KH_2PO_4, pH 7.5. The binding of the histamine H_1 antagonist [^3H]mepyramine (1nM, specific activity: 24.8 Ci/mM) was done in a final volume of 0.25 mL and incubated at room temperature for 60 min. Nonspecific binding was determined in the presence of 10 μM mepyramine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Histamine H_2 Receptor: The coding sequence of the human H_2 receptor was obtained from a human placenta genomic library, and cloned into the cloning site of PCEXV-3 eukaryotic expression vector. The plasmid DNA for the H_2 receptor is designated pcEXV-H2, and was deposited on November 6, 1992 under ATCC Accession No. 75345. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 min at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 min at 4 °C. The pellet was suspended in 37.8 mM $NaHPO_4$, 12.2 mM K_2PO_4, pH 7.5. The binding of the histamine H_2 antagonist [^3H]tiotidine (5nM, specific activity: 70 Ci/mM) was done in a final volume of 0.25 ml and incubated at room temperature for 60 min. Nonspecific binding was determined in the presence of 10 μM histamine. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human Serotonin Receptors:

$5HT_{1D\alpha}$, $5HT_{1D\beta}$, $5HT_{1E}$, $5HT_{1F}$ Receptors: LM(tk-) clonal cell lines stably transfected with the genes encoding each of these 5HT receptor subtypes were prepared as described above. The cell line for the $5HT_{1D\alpha}$ receptor, designated as Ltk-8-30-84, was deposited on April 17, 1990, and accorded ATCC Accession No. CRL 10421. The cell for the

$5HT_{1D\beta}$ receptor, designated as Ltk-11, was deposited on April 17, 1990, and accorded ATCC Accession No. CRL 10422. The cell line for the $5HT_{1E}$ receptor, designated 5 HT_{1E}-7, was deposited on November 6, 1991, and accorded ATCC Accession No. CRL 10913. The cell line for the $5HT_{1F}$ receptor, designated L-5-HT_{1F}, was deposited on December 27, 1991, and accorded ATCC Accession No. ATCC 10957. Membrane preparations comprising these receptors were prepared as described below, and suspended in 50mM Tris-HCl buffer (pH 7.4 at 37°C) containing 10 mM $MgCl_2$, 0.2 mM EDTA, 10μM pargyline, and 0.1% ascorbate. The binding of compounds was determined in competition binding assays by incubation for 30 minutes at 37°C in the presence of 5nM [^{3}H]serotonin. Nonspecific binding was determined in the presence of 10μM serotonin. The bound radioligand was separated by filtration through GF/B filters using a cell harvester.

Human $5HT_2$ Receptor: The coding sequence of the human $5HT_2$ receptor was obtained from a human brain cortex cDNA library, and cloned into the cloning site of pcEXV-3 eukaryotic expression vector. This construct was transfected into COS-7 cells by the DEAE-dextran method. Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. This cell line was deposited with the ATCC on October 31, 1989, designated as L-NGC-$5HT_2$, and was accorded ATCC Accession No. CRL 10287. The cell lysates were centrifuged at 1000 rpm for 5 minutes at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50mM Tris-HCl buffer (pH 7.7 at room temperature) containing 10 mM $MgSO_4$, 0.5mM EDTA, and 0.1% ascorbate. The potency of alpha-1 antagonists at $5HT_2$ receptors was determined in equilibrium competition binding assays using [3H]ketanserin (1nM). Nonspecific binding was defined by the addition of 10μM mianserin. The bound radioligand was separated by filtration through

GF/B filters using a cell harvester.

Human 5-HT$_7$ Receptor: A LM(tk-) clonal cell line stably transfected with the gene encoding the 5HT$_7$ receptor subtype was prepared as described above. The cell line for the 5HT$_7$ receptor, designated as L-5HT$_{4B}$, was deposited on October 20, 1992, and accorded ATCC Accession No. CRL 11166.

Human Dopamine D$_3$ Receptor: The binding of compounds to the human D3 receptor was determined using membrane preparations from COS-7 cells transfected with the gene encoding the human D$_3$ receptor. The human dopamine D3 receptor was prepared using known methods. Sokoloff, P. et al., Nature, 347, 146 (1990), and deposited with the European Molecular Biological Laboratory (EMBL) Genbank as X53944). Cells were harvested after 72 hours and lysed by sonication in 5mM Tris-HCl, 5mM EDTA, pH 7.5. The cell lysates were centrifuged at 1000 rpm for 5 minutes at 4°C, and the supernatant was centrifuged at 30,000 x g for 20 minutes at 4°C. The pellet was suspended in 50 mM Tris-HCl (pH 7.4) containing 1mM EDTA, 5mM KCl, 1.5mM $CaCl_2$, 4mM $MgCl_2$, and 0.1% ascorbic acid. The cell lysates were incubated with [^{3}H]spiperone (2nM), using 10μM (+)Butaclamol to determine nonspecific binding.

Membrane Harvest

Membranes were harvested from COS-7 cells 48 hours after transient transfection. Adherent cells were washed twice in ice-cold phosphate buffered saline (138 mM NaCl, 8.1 mM Na_2HPO_4, 2.5 mM KCl, 1.2 mM KH_2PO_4, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4) and lysed by sonication in ice-cold sonication buffer (20 mM Tris-HCl, 5 mM EDTA, pH 7.7). Large particles and debris were cleared by low speed centrifugation (200 x g, 5 min, 4°C). Membranes were collected from the supernatant fraction by centrifugation

(32,000 x g, 18 min, 4°C), washed with ice-cold hypotonic buffer, and collected again by centrifugation (32,000 x g, 18 min, 4°C). The final membrane pellet was resuspended by sonication into a small volume of ice-cold binding buffer (~1 mL for every 5 plates: 10 mM NaCl, 20 mM HEPES, 0.22 mM KH_2PO_4, 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4$, pH 7.4). Protein concentration was measured by the Bradford method (Bradford, 1976) using Bio-Rad Reagent, with bovine serum albumin as a standard. Membranes were held on ice for up to one hour and used fresh, or flash-frozen and stored in liquid nitrogen.

Membranes were prepared similarly from 293, LM(tk-), and NIH-3T3 cells. To prepare membranes from baculovirus infected cells, 2 x 10^7 Sf21 cells were grown in 150mm tissue culture dishes and infected with a high-titer stock of hY5BB3. Cells were incubated for 2-4 days at 27°C, no CO_2 before harvesting and membrane preparation as described above.

Membranes were prepared similarly from dissected rat hypothalamus. Frozen hypothalami were homogenized for 20 seconds in ice-cold sonication buffer with the narrow probe of a Virtishear homogenizer at 1000 rpm (Virtis, Gardiner, NY). Large particles and debris were cleared by centrifugation (200 x g, 5 min, 4°C) and the supernatant fraction was reserved on ice. Membranes were further extracted from the pellet by repeating the homogenization and centrifugation procedure two more times. The supernatant fractions were pooled and subjected to high speed centrifugation (100,000 x g, 20 min. 4°C). The final membrane pellet was resuspended by gentle homogenization into a small volume of ice-cold binding buffer (1 mL/ gram wet weight tissue) and held on ice for up to one hour, or flash-frozen and stored in liquid nitrogen.

Radioligand Binding to Membrane Suspensions

Membrane suspensions were diluted in binding buffer supplemented with 0.1% bovine serum albumin to yield an optimal membrane protein concentration so that ^{125}I-PYY (or alternative radioligand such as ^{125}I-NPY, ^{125}I-PYY$_{3-36}$, or ^{125}I-[Leu^{31}Pro^{34}]PYY) bound by membranes in the assay was less than 10% of ^{125}I-PYY (or alternative radioligand) delivered to the sample (100,000 dpm/sample = 0.08 nM for competition binding assays). ^{125}I-PYY (or alternative radioligand) and peptide competitors were also diluted to desired concentrations in supplemented binding buffer. Individual samples were then prepared in 96-well polypropylene microtiter plates by mixing ^{125}I-PYY (25 μL) (or alternative radioligand), competing peptides or supplemented binding buffer (25 μL), and finally, membrane suspensions (200 μl). Samples were incubated in a 30°C water bath with constant shaking for 120 min. Incubations were terminated by filtration over Whatman GF/C filters (pre-coated with 1% polyethyleneimine and air-dried before use), followed by washing with 5 mL of ice-cold binding buffer. Filter-trapped membranes were impregnated with MultiLex solid scintillant (Wallac, Turku, Finland) and counted for ^{125}I in a Wallac Beta-Plate Reader. Non-specific binding was defined by 300 nM human NPY for all receptors except the Y4 subtypes; 100 nM human PP was used for the human Y4 and 100 nM rat PP for the rat Y4. Specific binding in time course and competition studies was typically 80%; most non-specific binding was associated with the filter. Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

The canine Y5 receptor pharmacology was investigated using porcine ^{125}I-PYY as described above. Nonspecific binding was defined by 1 μM human NPY. As above, membranes were collected by filtration over Whatman GF/C

filters and counted for radioactivity.

Functional Assay: Radioimmunoassay of cAMP

Stably transfected cells were seeded into 96-well microtiter plates and cultured until confluent. To reduce the potential for receptor desensitization, the serum component of the media was reduced to 1.5% for 4 to 16 hours before the assay. Cells were washed in Hank's buffered saline, or HBS (150 mM NaCl, 20 mM HEPES, 1 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM glucose) supplemented with 0.1% bovine serum albumin plus 5 mM theophylline and pre-equilibrated in the same solution for 20 min at 37°C in 5% CO_2. Cells were then incubated 5 min with 10 μM forskolin and various concentrations of receptor-selective ligands. The assay was terminated by the removal of HBS and acidification of the cells with 100 mM HCl. Intracellular cAMP was extracted and quantified with a modified version of a magnetic bead-based radioimmunoassay (Advanced Magnetics, Cambridge, MA). The final antigen/antibody complex was separated from free ^{125}I-cAMP by vacuum filtration through a PVDF filter in a microtiter plate (Millipore, Bedford, MA). Filters were punched and counted for ^{125}I in a Packard gamma counter. Binding data were analyzed using nonlinear regression and statistical techniques available in the GraphPAD Prism package (San Diego, CA).

Functional Assay: Intracellular calcium mobilization

The intracellular free calcium concentration was measured by microspectroflourometry using the fluorescent indicator dye Fura-2/AM (ref). Stably transfected cells were seeded onto a 35 mm culture dish containing a glass coverslip insert. Cells were washed with HBS and loaded with 100 μl of Fura-2/AM (10 μM) for 20 to 40 min. After washing with HBS to remove the Fura-2/AM solution, cells were equilibrated in HBS for 10 to 20 min. Cells were then visualized under the 40X objective of a Leitz

Fluovert FS microscope and fluorescence emission was determined at 510 nM with excitation wave lengths alternating between 340 nM and 380 nM. Raw fluorescence data were converted to calcium concentrations using standard calcium concentration curves and software analysis techniques.

Tissue preparation for neuroanatomical studies

Male Sprague-Dawley rats (Charles Rivers) were decapitated and the brains rapidly removed and frozen in isopentane. Coronal sections were cut at 11 μm on a cryostat and thaw-mounted onto poly-L-lysine coated slides and stored at -80°C until use. Prior to hybridization, tissues were fixed in 4% paraformaldehyde, treated with 5 mM dithiothreitol, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, delipidated with chloroform, and dehydrated in graded ethanols.

Probes

The oligonucleotide probes employed to characterize the distribution of the rat NPY Y5 mRNA were complementary to nucleotides 1121 to 1165 in the 5,6-loop of the rat Y5 mRNA (Fig. 3A) 45mer antisense and sense oligonucleotide probes were synthesized on a Millipore Expedite 8909 Nucleic Acid Synthesis System. The probes were then lyophilized, reconstituted in sterile water, and purified on a 12% polyacrylamide denaturing gel. The purified probes were again reconstituted to a concentration of 100 ng/μL, and stored at -20°C.

In Situ Hybridization

Probes were 3'-end labeled with ^{35}S-dATP (1200 Ci/mmol, New England Nuclear, Boston, MA) to a specific activity of 10^9 dpm/μg using terminal deoxynucleotidyl transferase (Pharmacia). The radiolabeled probes were purified on Biospin 6 chromatography columns (Bio-Rad; Richmond, CA),

and diluted in hybridization buffer to a concentration of 1.5×10^4 cpm/μL. The hybridization buffer consisted of 50% formamide, 4X sodium citrate buffer (1X SSC = 0.15 M NaCl and 0.015 M sodium citrate), 1X Denhardt's solution (0.2% polyvinylpyrrolidine, 0.2% Ficoll, 0.2% bovine serum albumin), 50 mM dithiothreitol, 0.5 mg/ml salmon sperm DNA, 0.5 mg/ml yeast tRNA, and 10% dextran sulfate. One hundred μL of the diluted radiolabeled probe was applied to each section, which was then covered with a Parafilm coverslip. Hybridization was carried out overnight in humid chambers at 40 to 55°C. The following day the sections were washed in two changes of 2X SSC for one hour at room temperature, in 2X SSC for 30 min at 50-60°C, and finally in 0.1X SSC for 30 min at room temperature. Tissues were dehydrated in graded ethanols and exposed to Kodak XAR-5 film for 3 days to 3 weeks at -20°C, then dipped in Kodak NTB2 autoradiography emulsion diluted 1:1 with 0.2% glycerol water. After exposure at 4°C for 2 to 8 weeks, the slides were developed in Kodak D-19 developer, fixed, and counterstained with cresyl violet.

Hybridization controls

Controls for probe/hybridization specificity included hybridization with the radiolabeled sense probe, and the use of transfected cell lines. Briefly, COS-7 cells were transfected (see above) with receptor cDNAs for the rat Y1, Y2 (disclosed in US patent application 08/192,288, filed February 3, 1994), Y4 (disclosed in US patent application 08/176,412, filed December 28, 1993), or Y5. As described above, the transfected cells were treated and hybridized with the radiolabeled Y5 antisense and sense oligonucleotide probes, washed, and exposed to film for 1-7 days.

Analysis of hybridization signals

Sections through the rat brain were analyzed for

hybridization signals in the following manner. "Hybridization signal" as used in the present context indicates the relative number of silver grains observed over neurons in a selected area of the rat brain. Two independent observers rated the intensity of the hybridization signal in a given brain area as nonexistent, low, moderate, or high. These were then converted to a subjective numerical scale as 0, +1, +2, or +3 (see Table 10), and mapped on to schematic diagrams of coronal sections through the rat brain (see Fig. 11).

Chemical synthetic methods

Compounds evaluated in the *in vitro* Y5 receptor binding and functional assays, and *in vivo* feeding assays of the present invention (*infra*) were synthesized according to the methods described below. Binding of the compounds to the human Y1, Y2, Y4 and Y5 receptors was evaluated using stably transfected 293 or LM(tk-) cells as described above, except that the binding data reported for compound 1 at the human Y1 and Y2 receptors also included data derived from transiently transfected COS-7 cells. Stably transfected cell lines which may be used for binding experiments include, for the Y1 receptor, 293-hY1-5 (deposited June 4, 1996, under ATCC Accession No. CRL-12121); for the Y2 receptor, 293-hY2-10 (deposited January 27, 1994, under ATCC Accession No. CRL-11837); for the Y4 receptor, L-hY4-3 (deposited January 11, 1995, under ATCC Accession No. CRL 11779); and for the Y5 receptor, L-hY5-7 (deposited November 15, 1995, under ATCC Accession No. CRL 11995).

It is generally preferred that the respective product of each process step, as described hereinbelow, is separated and/or isolated prior to its use as starting material for subsequent steps. Separation and isolation can be effect by any suitable purification procedure such as, for example, evaporation, crystallization, column

chromatography, thin layer chromatography, distillation, etc. While preferred reactants have been identified herein, it is further contemplated that the present invention would include chemical equivalents to each reactant specifically enumerated in this disclosure.

Temperatures are given in degrees Centigrade (°C). The structure of final products, intermediates and starting materials is confirmed by standard analytical methods, e.g., microanalysis and spectroscopic characteristics (e.g. MS, IR, NMR). Unless otherwise specified, chromatography is carried out using silica gel. Flash chromatography refers to medium pressure column chromatography according to Still et al., J. Org. Chem. 43, 2928 (1978).

Synthesis of Compounds 1, 2, 5, 6, 7, 9, 10, and 11

For Compounds 1, 2, 5, 6, 7, 9, 10, and 11, thin layer chromatography was performed using the following solvent system:

A1:	dichloromethane/methanol	9:1
A2:	dichloromethane/methanol	19:1
A3:	dichloromethane/methanol/ammonium hydroxide	90:10:1
B1:	toluene/ethylacetate	1:1
B2:	toluene/ethylacetate	10:1
C1:	hexanes/ethylacetate	4:1
C2:	hexanes/ethylacetate	3:1
C3:	hexanes/ethylacetate	2:1

Compound 1: 2,4-Diphenylamino-quinazoline hydrochloride

2-Chloro-4-phenylamino-quinazoline (7.671 g) and aniline (3.627 g) are heated for 3 min to produce a melt which is dissolved in methanol. The product is obtained as its hydrochloride salt upon addition of a slight excess of 4N HCl in dioxane. Recrystallization from isopropanol yields 2,4-diphenylamino-quinazoline hydrochloride, m.p. 319 - 320°C, FAB-MS (Fast Atom Bombardment Mass Spectroscopy): $(M+H)^+ = 313$. Analytical data: $C_{20}H_{16}N_4$ + HCl + 0.5 H_2O, m.p. 319-320°C.

The starting material can be prepared as follows:

a) 2-Chloro-4-phenylamino-quinazoline

A solution of 2,4-dichloro-quinazoline (15 g), N,N-diisopropyl-ethylamine (24.9 ml) and aniline (7.5 ml) in isopropanol (75 ml) is heated to reflux for 45 min. The cold reaction mixture is filtered and the filtrate is concentrated *in vacuo*. The residue is crystallized from diethylether- toluene (1:1) to give 2-chloro-4-phenyl-amino-quinazoline, m.p. 194 - 196°C.

b) 2,4-Dichloro-quinazoline

N,N-Dimethylaniline (114.0 g) is added slowly to a solution of 1H,3H-quinazolin-2,4-dione (146.0 g) in phosphorousoxychloride (535.4 ml) while this mixture is heated up to 140°C. After completion of the addition reflux is continued for 20 h. The reaction mixture is filtered and evaporated to give a residue which is added to ice and water. The product is extracted with dichloromethane and crystallized from diethylether and petroleum diethylether to yield 2,4-dichloro-quinazoline, m.p. 115 - 116°C.

Compound 2: Naphthalene-1-sulfonic acid [6-(4-amino-quinazolin-2-ylamino)-hexyl]-amide

A solution of naphthalene-1-sulfonic acid (6-amino-hexyl)-amide (0.450 g) and 2-chloro-quinazolin-4-ylamine (see: US 3,956,495) (0.264 g) in 20 ml of isopentylalcohol is heated up to 120°C for 15 h. Concentration of the reaction mixture followed by chromatography on silica gel (B1) yields naphthalene-1-sulfonic acid [6-(4-amino-quinazolin-2-ylamino)-hexyl]-amide as a white powder, melting at 98-101°C. Rf(B1) 0.28, FAB-MS: $(M+H)^+ = 450$. Analytical $C_{26}H_{29}N_5O_2S$ + HCl + H_2O + 0.6 1,4 dioxane. m.p. 98-101 °C.

Compound 5: *trans*-Naphthalene-1-sulfonic acid {4-[(4-amino-quinazolin-2-ylamino)-methyl]-cyclohexylmethyl}-

amide hydrochloride

A suspension of 2-chloro-quinazolin-4-ylamine (7.02 g) and *trans*-naphthalene-1-sulfonic acid (4-aminomethyl-cyclohexylmethyl)-amide (13 g) in 250 ml of isopentyl-alcohol is heated up to 120°C for 15 h. The resulting solution is concentrated and chromatographed (silica gel, B2) to give the product as a foam. This material is taken up in dichloromethane (250 ml) and treated at 0°C with a 4 N HCl solution in dioxane (10 ml). Concentration *in vacuo* provides a foam which is triturated in boiling cyclohexane to yield after filtration *trans*-naphthalene-1-sulfonic acid {4-[(4-amino-quinazolin-2-ylamino)-methyl]-cyclohexylmethyl}-amide hydrochloride melting at 155 - 164°C. Rf(B2) 0.23, FAB-MS: $(M+H)^+$ = 476. m.p. 155-164 °C.

The starting material is prepared as follows:

a) *trans*-(4-Hydroxymethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester

A solution of *trans*-4-(*tert*-butoxycarbonylamino-methyl)-cyclohexanecarboxylic acid (obtained according to: EP 0614 911 A1) (34.5 g) and triethylamine (28 ml) in dichloromethane (700 ml) is cooled to -70°C and treated with methylchloroformate (12.9 ml). The reaction mixture is stirred 0.5 h at -70°C. The temperature is allowed to increase to 0°C and the solution is stirred another 0.5 h until completion of the reaction. The reaction mixture is taken up in ice-cold dichloromethane, washed with an ice-cold 0.5 N HCl solution, a saturated aqueous sodium carbonate solution and water. The organics are dried over sodium sulfate and concentrated to 41.3 g of mixt-anhydride as an oil. This material is taken up in THF and treated at -70°C with sodium borohydride (5.90 g), followed by absolute methanol (10 ml). The reaction mixture is stirred 15 h at 0°C and 1 h at ambient temperature to drive the reaction to completion. A 0.5N HCl solution is then carefully added at 0°C, followed by

ethyl acetate. The organics are washed with a saturated aqueous sodium carbonate solution, water, dried over sodium sulfate and concentrated. Chromatography on silica gel (A1) yields *trans*-(4-hydroxymethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester as a white powder, melting at 88 - 89°C. Rf(A1) 0.24.

b) *trans*-(4-Azidomethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester

trans-(4-Hydroxymethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester (24 g) in pyridine (200 ml) at 0°C is treated with a solution of *para*-toluenesulfonylchloride (24.44 g) in pyridine (50 ml). The reaction mixture is stirred at 0°C until completion and concentrated *in vacuo*. The residue is taken up in ethyl acetate, washed with water and dried over sodium sulfate. Concentration of the solution yields the tosylate, used without further purification. This material is treated with sodium azide (19.23 g) in N,N-dimethylformamide (800 ml) at 50°C. After completion of the reaction, the solution is concentrated and the resulting paste is taken up in dichloromethane, washed with water and concentrated. Chromatography of the crude material on silica gel (A2 then A3) provides *trans*-(4-azidomethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester as an oil. Rf(A3) 0.33; IR (dichloromethane) λ max 2099 cm^{-1}.

c) *trans*-(4-Aminomethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester

trans-(4-Azidomethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester (24 g) in ethyl acetate (1 liter) is hydrogenated over platinumoxide (2.4 g) at ambient temperature under atmospheric pressure of hydrogen. The catalyst is filtered-off and the filtrate concentrated to yield *trans*-(4-aminomethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester as an oil. Rf(C2) 0.41.

d) *trans*-{4-[(Naphthalene-1-sulfonylamino)-methyl]-cyclohexylmethyl}-carbamic acid *tert*-butyl ester

A solution of *trans*-(4-aminomethyl-cyclohexylmethyl)-carbamic acid *tert*-butyl ester (17 g) and ethyldiisopropylamine (14.41 ml) in N,N-dimethylformamide (350 ml) is cooled to 0°C and treated with a solution of naphthalene-1-sulfonylchloride (15.9 g) in N,N-dimethylformamide (100 ml). The reaction is stirred at ambient temperature for 2 h, concentrated *in vacuo*. The residue is taken up in dichloromethane, washed with a 0.5 N HCl solution, a saturated aqueous sodium carbonate solution and water, dried and concentrated. Crystallization from hexanes-ethyl acetate gives *trans*-{4-[(naphthalene-1-sulfonylamino)-methyl]-cyclohexylmethyl}-carbamic acid *tert*-butyl ester as a white powder, melting at 199 - 200°C. Rf(A1) 0.42.

e) *trans*-Naphthalene-1-sulfonic acid (4-aminomethyl-cyclohexylmethyl)-amide

A suspension of *trans*-{4-[(naphthalene-1-sulfonylamino)-methyl]-cyclohexylmethyl}-carbamic acid *tert*-butyl ester (25 g) in chloroform (300 ml) is treated with a 4 N HCl solution in dioxane (300 ml) at 0°C. After completion, the reaction mixture is concentrated *in vacuo*, the residue is taken up in a 1 N sodium hydroxide solution and dichloromethane. After extraction with dichloromethane, the organics are dried over sodium sulfate and concentrated to 18.5 g of *trans*-naphthalene-1-sulfonic acid (4-aminomethyl-cyclohexylmethyl)-amide as a white powder melting at 157 - 162°C. Rf(C3) 0.36.

<u>Compound 6: 2-[4-(Piperidin-1-yl)-phenylamino]-4-phenylamino-quinazoline dihydrochloride</u>

A mixture of 2-chloro-4-phenylamino-quinazoline (0.18 g) and N-(4-aminophenyl)-piperidine (0.164 g) is heated for 3 min to produce a melt which is dissolved in isopropanol (4 ml). 4 N HCl in dioxane (1 ml) is added.

Recrystallization from ethanol and diethylether yields 2-[4-(piperidin-1-yl)-phenylamino]-4-phenylamino-quinazoline dihydrochloride, Rf (A1) 0.64, FAB-MS: $(M+H)^+$ = 396. m.p.: (decomposition).

Compound 7: *trans*-2-(4-Acetoxy-cyclohexylamino)-4-phenylamino-quinazoline hydrochloride

A solution of *trans*-2-(4-hydroxy-cyclohexyamino)-4-phenylamino-quinazoline hydrochloride (1.3 g) and acetic anhydride (0.33 ml) in acetic acid (5 ml) is stirred at ambient temperature for 16 h. The solvent is removed *in vacuo* and the residue is added to 2N aqueous NaOH. Extraction with ethyl acetate followed by chromatography on silica gel (A4) gives a crude product which is treated with 4 N HCl in dioxane. Crystallization from acetonitrile and acetone yields *trans*-2-(4-acetoxy-cyclohexylamino)-4-phenylamino-quinazoline hydrochloride, m.p. 217 - 220°C; FAB-MS: $(M+H)^+$ = 377; analytical data: $C_{22}H_{24}N_4O_2$ + HCl.

The starting material is prepared as follows:

a) 2-(4-Hydroxy-cyclohexyamino)-4-phenylamino-quinazoline hydrochloride

A mixture of 2-chloro-4-phenylamino-quinazoline (2.3 g) and *trans*-4-amino-cyclohexanol (1.26 g) is heated for 3 min to produce a melt which is dissolved in isopropanol. 4 N HCl in dioxane (0.1 ml) is added. Crystallization from isopropanol and acetone yields 2-(4-hydroxy-cyclohexyamino)-4-phenylamino-quinazoline hydrochloride, m.p. 258 - 259°C.

Compound 9: 8-Methoxy-2-(4-methoxy-phenylamino)-4-phenylamino-quinazoline hydrochloride

A mixture of 2-chloro-8-methoxy-4-phenylamino-quinazoline (1.20 g) and 4-methoxy-aniline (0.66 g) is heated for 3 min to produce a melt which is dissolved in isopropanol (15 ml). 4N HCl in dioxane (0.2 ml) is added.

Crystallization from isopropanol and diethylether yields 8-methoxy-2-(4-methoxy-phenylamino)-4-phenylamino-quinazoline dihydrochloride, m.p. 287 - 289°C, FAB-MS: $(M+H)^+ = 373$. Analytical data: $C_{22}H_{20}N_4O_2$ + HCl.

The starting material can be prepared as follows:

a) 2-Chloro-8-methoxy-4-phenylamino-quinazoline

A solution of 2,4-dichloro-8-methoxy-quinazoline (prepared as described in *J. Chem. Soc.* **1948**, 1759) (0.6 g), N,N-diisopropyl-ethylamine (0.87 ml), and aniline (0.26 ml) in isopropanol (10 ml) is heated to reflux for 45 min. The cold reaction mixture is filtered and residue is crystallized from dichloromethane and hexanes to give 2-chloro-8-methoxy-4-phenylamino-quinazoline, m.p. 245 - 246°C.

<u>Compound 10: N-Methyl-[4-(6-methoxy-4-phenylamino-quinazolin-2-ylamino)-phenyl]-methanesulfonamide hydrochloride</u>

A solution of 2-chloro-6-methoxy-4-phenylamino-quinazoline (1.15 g) and N-methyl-(4-aminophenyl)-methanesulfonamide (prepared as described in *Tetrahedron Letters* **1992,** *33*, 8011) (0.89 g) in 5 mL of isopentylalcohol is stirred under nitrogen at 180°C for 20 min in a sealed vessel. The warm reaction mixture is diluted with methanol and the hydrochloride salt, which is crystallizing on cooling, is filtered off. The crude product is redissolved in ethylacetate and aqueous sodium carbonate solution and extracted with ethylacetate. The organic extracts are dried and evaporated and the solid residue is titurated with diethylether to give N-methyl-[4-(6-methoxy-4-phenylamino-quinazolin-2-ylamino)-phenyl]-methanesulfonamide as light yellow crystals melting at 212 - 215°C; (Rf (A2) 0.16. Recrystallisation from methanolic hydrogen chloride and diethylether yields N-methyl-[4-(6-methoxy-4-phenylamino-quinazolin-2-ylamino)-phenyl]-methanesulfonamide hydrochloride as

light yellow crystals melting at 264 - 268°C; Rf (A2) 0.16, FAB-MS: $(M+H)^+$ = 450. Analytical data: $C_{23}H_{23}N_5O_3S$ + HCl.

The starting material can be prepared as follows:

a) 2-Chloro-6-methoxy-4-phenylamino-quinazoline

In a procedure analogous to that of Example 1a 2,4-dichloro-6-methoxy-quinazoline (1.53 g) (prepared as described in *J. Chem. Soc.* **1948**, 1759), aniline (0.8 g) (0.184 g) and N,N-diisopropyl-ethylamine (1.72 g) are reacted together to give 2-chloro-6-methoxy-4-phenylamino-quinazoline as light yellow crystals melting at 177 - 179°C, Rf (A2) 0.59.

<u>Compound 11: N-Methyl-[4-(4-phenylamino-quinazolin-2-ylamino)-phenyl]-methanesulfonamide hydrochloride</u>

A solution of 2-chloro-4-phenylamino-quinazoline (0.92 g) (prepared as described in Example 1a and N-methyl-(4-aminophenyl)-methanesulfonamide (0.80 g) in 10 ml of isopentylalcohol is stirred under nitrogen at 170°C for 15 min in a sealed vessel. The warm reaction mixture is diluted with 10 ml ethanol and the hydrochloride salt, which is crystallizing on cooling, is filtered off to yield N-methyl-[4-(4-phenylamino-quinazolin-2-ylamino)-phenyl]-methanesulfonamide hydrochloride as light yellow crystals melting at 259 - 263°C; Rf (A2) 0.11, FAB-MS: $(M+H)^+$ = 420. Analytical data: $C_{22}H_{21}N_5O_2S$ + HCl.

<u>Synthesis of Compounds 17-23, Compound 26 and Compound 27.</u>

Compounds 17-23, 26 and 27 were synthesized according to the general method in Scheme 1, as described below. An example of the synthesis of a specific compound, Compound 17, follows the general description. Compounds 18-23, 26 and 27 were synthesized in the same manner but using the appropriately substituted starting materials.

Preparation of the compounds of the present invention having the structure shown in Formula 1-3, Scheme 1, is carried out using well-known methodology for the preparation of a sulfonamide from an amine. Preferably the appropriate arylsulfonyl halide, preferably the chloride (i.e., $Ar-SO_2Cl$), is reacted with a monoprotected linear or cyclic alkylamine (Krapcho and Kuell, Synth. Comm. 20(16):2559-2564, 1990) comprising H_2N-L-K'', where K'' comprises methylene, in the presence of a base such as a tertiary amine, e.g., triethylamine, dimethylaminopyridine, pyridine or the like, in an appropriate solvent (e.g. $CHCl_3$, CH_2Cl_2) as shown in Scheme 1, step A, followed by deprotection of the resulting amine as shown in Scheme 1, Step B, all under mild conditions (typically room temperature), to yield the deprotected amine of Formula 1-1. The arylsulfonyl halides are either known in the art or can be prepared according to methods well known in the art.

Compounds of Formula 1-2 in Scheme 1, may be synthesized from the compound of Formula 1-1 by amidation using suitable methods such as those taught in "The Peptides," Vol. 1 (Gross and Meinehofer, Eds. Acaemic Press, N.Y., 1979). For example, the compound of Formula 1-1 may be treated with a carboxylic acid derivative of W in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and dimethylaminopyridine (DMAP) in a suitable solvent such as CH_2Cl_2 as shown in Scheme 1, Step C, at room temperature in an inert atmosphere of argon or nitrogen, to yield the amide compound of Formula 1-2. The K'' amine and the carboxylic acid carbon attached to W together form K in the product.

Alternatively, the compound of Formula 1-2 may be synthesized by acylation of the amine of Formula 1-1 using the acid chloride of W, i.e., WCOCl, in a solvent such as CH_2Cl_2 and a suitable tertiary amine such as

triethylamine, at room temperature. Again, the K'' amine and the acid chloride carbon attached to W together form K in the product.

The product compounds of Formula 1-3 are then formed by reduction of the amide of Formula 1-3 using borane-tetrahydorfuran (THF) complex, in THF as shown in Scheme 1, Step D, at elevated temperature in an inert atmosphere.

Scheme 1

$$\text{Ar-SO}_2\text{Cl} \quad + \quad \text{H}_2\text{N-L-K''-NHBoc} \xrightarrow[\text{B. 1N HCl in Ether or EtOAc with HCl}]{\text{A. CH}_2\text{Cl}_2\text{, Et}_3\text{N}} \underset{\textbf{1-1}}{\text{Ar-SO}_2\text{-NH-L-K''-NH}_2}$$

$$\underset{\textbf{1-1}}{\text{Ar-SO}_2\text{-NH-L-K''-NH}_2} \xrightarrow[\text{or WCOCl, Et}_3\text{N, CH}_2\text{Cl}_2]{\text{C. W-CO}_2\text{H, EDC, DMAP, CH}_2\text{Cl}_2} \underset{\textbf{1-2}}{\text{Ar-SO}_2\text{-NH-L-K''-NH-CO-W}}$$

$$\underset{\textbf{1-2}}{\text{Ar-SO}_2\text{-NH-L-K''-NH-CO-W}} \xrightarrow{\text{D. BH}_3\text{.THF}} \underset{\textbf{1-3}}{\text{Ar-SO}_2\text{-NH-L-K-W}}$$

As a specific example of the synthesis of compounds 17-23, 26 and 27, the synthesis of Compound 17 is given hereinbelow.

Compound 17: Naphthalene-2-sulfonic acid(4-[{(1, 2, 3, 4-tetrahydronaphthalen-2-yl)methyl}-amino]-trans-yclohexylmethyl)-amide

Step A Scheme 1

{4-[(Naphthalene-2-sulfonylamino)-trans-cyclohexylmethyl]-carbamic acid tert-butyl ester:

To a stirred solution of (4-aminomethyl-cyclohexylmethyl)carbamic acid tert-butyl ester (0.50 g, 2.1 mmol) and triethyl amine (0.42 g, 4.2 mmol) in 50 mL methylene chloride was added 2-naphthalenesulfonyl chloride (0.51g, 2.3 mmol). The reaction mixture was stirred for 6 h at room temperature, quenched with brine, and extracted with methylene chloride (2x50 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo to yield the titled compound as white solid (0.74 g, 83%): mp 114-5°C.

Step B, Scheme 1

Naphthalene-2-sulfonic acid-(4-aminomethyl-trans-cyclohexylmethyl)-amide:

To a stirred solution of {4-[(naphthalene-2-sulfonylamino)-trans cyclohexylmethyl]-carbamic acid tert-butyl ester (0.73 g, 1.6 mmol) in 25 mL of methylene chloride at room temperature was added 3 mL of saturated HCl solution in ethyl acetate and stirred for 4 h. The precipitated solid was filtered to yield the titled compound as white solid (0.58 g, 99%); mp 286-7°C.

Step C, Scheme 1

1, 2, 3, 4-Tetrahydronaphthalene-2-carboxylic acid[4-{(naphthalen-2-sulfonylamino)methyl}-tans-cyclohexylmethyl]amide

A mixture of naphthalene-2-sulfonic acid-(4-aminomethyl-trans- cyclohexylmethyl)amide (0.5 g, 1.4 mmol), EDC (0.54 g, 2.8 mmol), and DMAP (0.34 g, 2.8 mmol) in methylene chloride(30 mL) was stirred at room temperature for 0.5h. 1,2,3,4-tetrahydronaphthalen-2-carboxylic acid (0.24 g, 1.4 mmol) was added to the reaction mixture and stirred at room temperature till the completion of the reaction (by TLC). The reaction mixture was washed with saturated ammonium chloride (3x30 mL), dried over sodium sulfate and concentrated in vacuo. The residue was flash chromatographed over silica gel to afford white solid (0.66 g, 99%); mp 225-6°C.

Step D, Scheme 1

Naphthalene-2-sulfonic acid(4-[{(1, 2, 3, 4-tetrahydronaphthalen-2-yl)methyl}-amino]-trans-cyclohexylmethyl)-amide

To a solution of 1, 2, 3, 4-tetrahydronaphthalen-2-carboxylic acid[4-{(naphthalen-2-sulfonylamino)methyl}-tanscyclohexylmethyl]amide(0.65 g, 1.3 mmol) in tetrahydrofuran (5 mL) cooled to 0°C was added 6.6 mL 1M solution of borane:THF complex and the reaction mixture

was refluxed for 12h. The reaction mixture was cooled in ice bath and quenched with 2 mL of 1N HCl. The reaction mixture was neutralized with 10% aqueous sodium hydroxide solution and extracted with ethyl acetate (3x25 mL). Organic phase was washed with the brine, dried over sodium sulfate, evaporated in vacuo to afford an oil which was purified by preparative TLC to afford the titled compound(0.44 g,70%); hydrochloride salt mp (210°C).

In order to synthesize compounds 18-24, 26 and 27, the 2-naphthalenesulfonyl chloride of Step A above, which comprises the "Ar" moiety of Table 2, is replaced with the appropriate Ar-sulfonyl chloride, and the 1,2,3,4-tetrahydronaphthalen-2-carboxylic acid used in Step C above, which comprises the "W" moiety of Table 2, is replaced with the appropriate W-carboxylic acid, to yield product containing the corresponding Ar and W moieties shown in Table 2.

Synthesis of Compound 25

Compound 25 was synthesized according to Scheme 2. After protection of H_2N-L-COOH with Boc anhydride in CH_2Cl_2, as shown in Scheme 2, Step A, the protected amine may be amidated with W-K''' as in Scheme 2, Step B, where K''' is $(CH_2)_jCHR_7$-NH_2, where R_7 is an ester and j is 1 using EDC and DMAP in a suitable solvent such as CH_2Cl_2, to yield compounds of Formula 3-1, where K''' and the carboxylic acid carbonyl of H_2N-L-COOH together form K. The compounds of Formula 3-1 may be deprotected using well known methods as shown in Scheme 2, Step C, and further sulfonylated with a sulfonyl halide of Ar, as shown in Scheme 2, Step D, in a suitable solvent such as CH_2Cl_2 and a tertiary amine such as triethylamine, to form the compound of Formula 3-3. Compounds of Formula 3-3 may be reduced to yield the compounds of Formula 3-3, as shown in Scheme 2, Step E, using borane-tetrahydorfuran

(THF) complex, in THF, at elevated temperature in an inert atmosphere.

Scheme 3

$$H_2N\text{-L-}CO_2H \xrightarrow[CH_2Cl_2]{\text{A. }(Boc)_2O} BocHN\text{-L-}CO_2H \xrightarrow[\text{EDC, DMAP, }CH_2Cl_2]{\text{B. W-}(CH_2)_j\text{-CHR7-}NH_2}$$

$$\underset{\textbf{3-1}}{BocHN\text{-L-CONHCHR7-}(CH_2)_j\text{-W}} \xrightarrow{\text{C. HCl, EtOAc}} \underset{\textbf{3-2}}{H_2N\text{-L-CONHCHR7-}(CH_2)_j\text{-W}}$$

$$\xrightarrow[CH_2Cl_2,\ Et_3N]{\text{D. }ArSO_2Cl} \underset{\textbf{3-3}}{ArSO_2HN\text{-L-K-W}}$$

Where $K = \text{-CONHCHR}_7(CH_2)_j\text{-}$

$$\underset{\textbf{3-3}}{ArSO_2HN\text{-L-CONHCHR7-}(CH_2)_j\text{-W}} \xrightarrow{\text{E. }BH_3\text{. THF}} \underset{\textbf{3-4}}{ArSO_2HN\text{-L-K-W}}$$

Where $K = \text{-}CH_2NHCHR_7(CH_2)_j\text{-}$

A detailed description of the synthesis of Compound 25 is given below:

Compound 25: trans-3-(4-Chloro-phenyl)-2-({[4-(naphthalene-1-sulfonylamino)-methyl]-cyclohexanecarbonyl}-amino]-propionic acid methyl ester:

(a) Step A, Scheme 2
trans-4-(tert-Butoxycarbonylamino-methyl)-cyclohexanecarboxylic acid:
To a solution of trans-4-(aminomethyl)cyclohexanecarboxylic acid (10 g, 57 mmol) in 1 N NaOH (110 mL) cooled to 0°C was added a solution of di-tert-butyl dicarbonate (15 g, 69 mmol) in dioxane

(50 mL). The reaction mixture was stirred at 0°C for 12 h. The reaction mixture was neutralized by 1 N HCl solution to pH 3, extracted with ethyl ether (2x300 mL), washed with brine (2x300 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford the titled compound (16 g, 100%); white solid, mp 128-9°C.

(b) Step B, Scheme 2

trans-2-{[4-(tert-Butoxycarbonylamino-methyl)-cyclohexanecarbonyl]-amino} 3-(4-Chloro-phenyl)-propionic acid methyl ester:

Using the general procedure described for the preparation Step B, Scheme 2, trans-4-(tert-butoxycarbonylamino-methyl)-cyclohexanecarboxylic acid (1.1 g, 4.0 mmol) was acylated with D,L-4-chlorophenylalanine methyl ester hydrochloride (1.0 g, 4.0 mmol) to afford the titled compound (1.9 g, 99%); white solid, mp 178-9°C.

(c) Step C, Scheme 2

trans-2-[4-(Aminomethyl-cyclohexanecarbonyl)-amino] 3-(4-chloro-phenyl)-propionic acid methyl ester hydrochloride:

Using the general procedure described in step **C** Scheme **2**, trans-2-{[4-(tert-butoxycarbonylamino-methyl)-cyclohexanecarbonyl]-amino} 3-(4-chloro-phenyl)-propionic acid methyl ester (1.8 g, 4.3 mmol) was deprotected using HCl in ethyl acetate to afford the titled compound; light yellow solid mp 146-9°C.

(d) Step D, Scheme 2

trans-3-(4-Chloro-phenyl)-2-({[4-(naphthalene-1-sulfonylamino)-methyl]-cyclohexanecarbonyl}-amino]-propionic acid methyl ester:

Using the general procedure described in step **B** Scheme 2, trans-2-[4-(aminomethyl-cyclohexanecarbonyl)-amino] 3-(4-Chloro-phenyl)-propionic acid methyl ester hydrochloride (0.35 g, 0.86 mmol) was sulfonylated with

1-naphthalenesulfonyl chloride (0.42 g, 91%) to afford the titled compound; white solid, mp 84-6°C.

Compound 25 was synthesized from the above compound by borane-THF reduction as follows:

(e) Step E, Scheme 2

Naphthalene-1-sulfonic Acid trans-(4-{[2-(4-Chloro-phenyl)-1-hydroxymethyl-ethylamino]-methyl}-cyclohexylmethyl)-amide:

Using the general procedure described in Step E, Scheme 2, trans-3-(4-chloro-phenyl)-2-({[4-(naphthalene-1-sulfonylamino)-methyl]-cyclohexanecarbonyl}-amino]-propionic acid methyl ester (0.30 g, 0.55 mmol) was reduced by borane:THF complex (1.0 M in THF) to afford the titled compound; colorless oil.

Synthesis of Compound 28

2-(Naphthalen-1-ylamino)-3-phenylpropionitrile

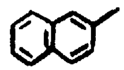
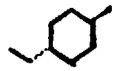
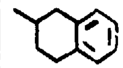
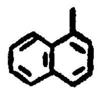
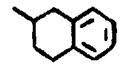
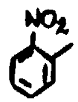
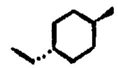
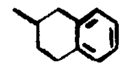
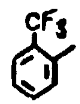
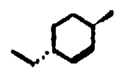
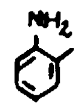
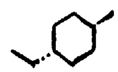
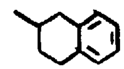
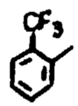
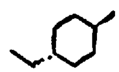
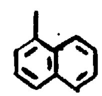
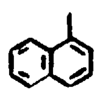
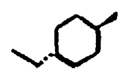
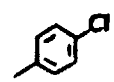
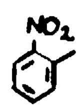
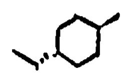
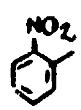
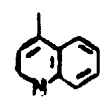
To a solution of 1-naphthalenemethylamine (2.9 g, 20 mmol) and benzylaldehyde (2.0 g, 17 mmol) in 30 ml of $CHCl_3$ and 10 ml of MeOH was added TMSCN (6.6 ml, 51 mmol) and the resulting solution was stirred for 12 h at 25°C. The reaction mixture was concentrated in vacuo, yielding an oil which was subjected to column chromatography (EtOAc, neat) to provide 3.5 g (74%) of the desired product as a colorless oil. Product was identified by NMR.

2-(Naphthalen-1-yl)-3-phenylpropane-1,2-diamine

To a solution of the nitrile (0.5 g, 1.8 mmol) in THF was added 6.9 ml of 1N $LiAlH_4$ in THF dropwise and the resulting solution was stirred for 2 h. The reaction was quenched by adding a few pieces of ice into the solution. The reaction mixture was diluted with EtOAc and filtered through pad of Celite. Organic filtrate was concentrated in vacuo to provide a oily residue which was subjected to

column chromatography (EtOAc, neat) to provide 0.28 g (57%) of the desired product as a colorless oil. The product was identified by NMR.

TABLE 2

No.	Ar	X	R_1	L	K	W	mp	Analysis
17		-	H		CH_2NHCH_2		210	$C_{29}H_{36}N_2O_2S$ + HCl
19		-	H		CH_2NHCH_2		220	$C_{29}H_{36}N_2O_2S$ + HCl + 0.15 CH_2Cl_2
20		-	H		CH_2NHCH_2		200-2	$C_{25}H_{33}N_3O_4S$ + HCl
21		-	H		CH_2NHCH_2		171-4	$C_{26}H_{29}N_2O_2SF_3$ + HCl + 0.075 $CHCl_3$
22		-	H		CH_2NHCH_2		175-7	$C_{25}H_{35}N_3O_2S$ + 2 HCl + 0.8 Et_2O
23		-	H		CH_2NHCH_2		216-7	$C_{26}H_{29}N_2O_2SF_3$ + HCl
25		-	H		$CH_2NHCH(CH_2OH)CH_2$		223-3	$C_{27}H_{33}N_2O_3SCl$ + HCl
26		-	H		CH_2NHCH_2		89 dec	$C_{24}H_{28}N_4O_4S$ + 2 HCl
27		-	H		CH_2NHCH_2		104-6	$C_{25}H_{28}N_4O_4S$ + 2 HCl + 0.2 $CHCl_3$

In vivo STUDIES IN RATS

Food intake in satiated rats

For these determinations food intake may be measured in normal satiated rats after intracerebroventricular application (i.c.v.) of NPY in the presence or absence of the test compound. Male Sprague Dawley rats (Ciba-Geigy AG, Sisseln, Switzerland) weighing between 180g and 220g are used for all experiments. The rats are individually housed in stainless steel cages and maintained on an 11:13 h light-dark cycle (lights off at 18:00 h) at a controlled temperature of 21-23 °C at all times. Water and food (NAFAG lab chow pellets NAFAG, Gossau, Switzerland) are available ad libidum.

Rats under pentobarbital anesthesia are stereotaxically implanted with a stainless steel guide cannula targeted at the right lateral ventricle. Stereotaxic coordinates, with the incisor bar set -2.0mm below interaural line, are: -0.8mm anterior and +1.3mm lateral to bregma. The guide cannula is placed on the dura. Injection cannulas extend the guide cannulas -3.8mm ventrally to the skull surface. Animals are allowed at least 4 days of recovery postoperatively before being used in the experiments. Cannula placement is checked postoperatively by testing all rats for their drinking response to a 50 ng intracerebroventricular (i.c.v.) injection of angiotensin II. Only rats which drink at least 2.5 ml of water within 30 min. after angiotensin II injection are used in the feeding studies.

All injections are made in the morning 2 hours after light onset. Peptides are injected in artificial cerebrospinal fluid (ACSF) in a volume of 5μl. ACSF contains: NaCl 124mM, KCl 3.75 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 2.0 mM, KH_2PO_4 0.22mM, $NaHCO_3$ 26 mM and glucose 10 mM. Porcine-NPY (p-NPY) are dissolved in artificial

cerebrospinal fluid (ACS). For i.c.v. injection the test compounds are preferably dissolved in DMSO/water (10%, v/v). The vehicle used for intraperitoneal (i.p.), subcutaneous (s.c.) or oral (p.o.) delivery of compounds is preferably water, physiological saline or DMSO/water (10% v/v), or cremophor/water (20% v/v), respectively.

Animals which are treated with both test compounds and porcine-NPY are treated first with the test compound. Then, 10 min. after i.c.v. application of the test compound or vehicle (control), or for i.p., s.c., or p.o. administration, 30-60 min after application of the test compound or vehicle, generally, NPY is administered by intracerebroventricular (i.c.v.) application.

Food intake may be measured by placing preweighed pellets into the cages at the time of NPY injection. Pellets are then removed from the cage subsequently at each selected time point and replaced with a new set of preweighed pellets. The food intake of animals treated with test compound may be calculated as a percentage of the food intake of control animals i.e., animals treated with vehicle. Alternatively, food intake for each group of animals subjected to a particular experimental condition may be expressed as the mean ± S.E.M. Statistical analysis is performed by analysis of variance using the Student-Newman-Keuls test.

Food intake in food-deprived rats

Food-deprivation experiments are conducted with male Sprague-Dawley rats weighing between 220g and 250g. After receipt, the animals are individually housed for the duration of the study and allowed free access to normal food together with tap water. The animals are maintained in a room with a 12 h light/dark cycle (8:00 a.m. to 8:00 p.m. light) at 24°C and monitored humidity. After placement into individual cages the rats undergo a

4 day equilibration period, during which they are habituated to their new environment and to eating a powdered or pellet diet NAFAG, Gossau, Switzerland).

At the end of the equilibration period, food is removed from the animals for 24 hours starting at 8:00 a.m. At the end of the fasting period compound or vehicle may be administered to the animals orally or by injection intraperitoneally or intravenously. After 10 - 60 min. food is returned to the animals and their food intake is monitored at various time periods during the following 24 hour period. The food intake of animals treated with test compound may be calculated as a percentage of the food intake of control animals (i.e., animals treated with vehicle). Alternatively, food intake for each group of animals subjected to a particular experimental condition may be expressed as the mean $\pm$ S.E.M.

Food intake in obese Zucker rats

The antiobesity efficacy of the compounds according to the present invention might also be manifested in Zucker obese rats, which are known in the art as an animal model of obesity. These studies are conducted with male Zucker fatty rats (fa/fa Harlan CPB, Austerlitz NL) weighing between 480g and 500g. Animals are individually housed in metabolism cages for the duration of the study and allowed free access to normal powdered food and water. The animals are maintained in a room with a 12 h light/dark cycle (light from 8:00 A.M. to 8:00 P.M.) at 24°C and monitored humidity. After placement into the metabolism cages the rats undergo a 6 day equilibration period, during which they are habituated to their new environment and to eating a powdered diet. At the end of the equilibration period, food intake during the light and dark phases is determined. After a 3 day control period, the animals are treated with test compounds or vehicle (preferably water or physiological saline or

DMSO/water (10%,v/v) or cremophor/water (20%,v/v)). Food intake is then monitored over the following 3 day period to determine the effect of administration of test compound or vehicle alone. As in the studies described hereinabove, food intake in the presence of drug may be expressed as a percentage of the food intake of animals treated with vehicle, or as the amount of food intake for a group of animals subjected to a particular experimental condition.

Materials

Cell culture media and supplements are from Specialty Media (Lavallette, NJ). Cell culture plates (150 mm and 96-well microtiter) were from Corning (Corning, NY). Sf9, Sf21, and High Five insect cells, as well as the baculovirus transfer plasmid, pBlueBacIII™, were purchased from Invitrogen (San Diego, CA). TMN-FH insect medium complemented with 10% fetal calf serum, and the baculovirus DNA, BaculoGold™, was obtained from Pharmingen (San Diego, CA.). Ex-Cell 400™ medium with L-Glutamine was purchased from JRH Scientific. Polypropylene 96-well microtiter plates were from Co-star (Cambridge, MA). All radioligands were from New England Nuclear (Boston, MA). Commercially available NPY and related peptide analogs were either from Bachem California (Torrance, CA) or Peninsula (Belmont, CA); [D-Trp^{32}]NPY and PP C-terminal fragments were synthesized by custom order from Chiron Mimotopes Peptide Systems (San Diego, CA). Bio-Rad Reagent was from Bio-Rad (Hercules, CA). Bovine serum albumin (ultra-fat free, A-7511) was from Sigma (St. Louis. MO). All other materials were reagent grade.

EXPERIMENTAL RESULTS

cDNA Cloning

In order to clone a rat hypothalamic "atypical" NPY receptor subtype, applicants used an expression cloning

strategy in COS-7 cells (Gearing et al, 1989; Kluxen et al, 1992; Kiefer et al, 1992). This strategy was chosen for its extreme sensitivity since it allows detection of a single "receptor positive" cell by direct microscopic autoradiography. Since the "atypical" receptor has only been described in feeding behavior studies involving injection of NPY and NPY related ligands in rat hypothalamus (see introduction), applicants first examined its binding profile by running competitive displacement studies of ^{125}I-PYY and ^{125}I-PYY$_{3-36}$ on membranes prepared from rat hypothalamus. The competitive displacement data indicate: 1) Human PP is able to displace 20% of the bound ^{125}I-PYY with an IC$_{50}$ of 11 nM (Fig. 1 and Table 3). As can be seen in Table 5, this value does not fit with the isolated rat Y1, Y2 and Y4 clones and could therefore correspond to another NPY/PYY receptor subtype. 2) [Leu$_{31}$, Pro$_{34}$] NPY (a Y1 specific ligand) is able to displace with high affinity (IC$_{50}$ of 0.38) 27% of the bound ^{125}I-PYY$_{3-36}$ ligand (a Y2 specific ligand) (Fig. 2 and Table 3). These data provide the first evidence based on a <u>binding assay</u> that rat hypothalamic membranes could carry an NPY receptor subtype with a mixed Y1/Y2 pharmacology (referred to as the "atypical" subtype) which fits with the pharmacology defined in feeding behavior studies.

TABLE 3: Pharmacological profile of the rat hypothalamus.

Binding data reflect competitive displacement of ^{125}I-PYY and ^{125}I-PYY$_{3-36}$ from rat hypothalamic membranes. Peptides were tested at concentrations ranging from 0.001 nM to 100 nM unless noted. The IC_{50} value corresponding to 50% displacement, and the percentage of displacement relative to that produced by 300 nM human NPY, were determined by nonlinear regression analysis. Data shown are representative of at least two independent experiments.

TABLE 3

Peptide	IC_{50} Values, nM (% NPY-produced displacement)	
	^{125}I-PYY	^{125}I-PYY$_{3-36}$
human NPY	0.82 (100%)	1.5 (100%)
human NPY$_{2-36}$	2.3 (100%)	1.2 (100%)
human [Leu^{31},Pro^{34}]NPY	0.21 (44%) 340 (56%)	0.38 (27%) 250 (73%)
human PYY	1.3 (100%)	0.29 (100%)
human PP	11 (20%)	untested

Based on the above data, a rat hypothalamic cDNA library of 3 x 10^6 independent recombinants with a 2.7 kb average insert size was fractionated into 450 pools of ≈7500 independent clones. All pools were tested in a binding assay with ^{125}I-PYY as previously described (US Serial No. 08/192,288). Seven pools gave rise to positive cells in the screening assay (#'s 81, 92, 147, 246, 254, 290, 312). Since Y1, Y2, Y4 and Y5 receptor subtypes (by PCR or binding analysis) are expressed in rat hypothalamus, the DNA of positive pools were analyzed by PCR with rat

Y1, Y2 and Y4 specific primers. Pools # 147, 246, 254 and 312 turned out to contain cDNAs encoding a Y1 receptor; pool # 290 turned out to contain cDNA encoding a Y2 receptor subtype; but pools # 81 and 92 were negative by PCR analysis for Y1, Y2 and Y4 and therefore likely contained a cDNA encoding a new rat hypothalamic NPY receptor (Y5). Pools # 81 and 92 later turned out to contain an identical NPY receptor cDNA. Pool 92 was subjected to sib selection until a single clone was isolated (designated CG-18).

The isolated clone carries a 2.8 kb cDNA. This cDNA contains an open reading frame between nucleotides 779 and 2146 that encodes a 456 amino acid protein. The long 5′ untranslated region could be involved in the regulation of translation efficiency or mRNA stability. The flanking sequence around the putative initiation codon does not conform to the Kozak consensus sequence for optimal translation initiation (Kozak, 1989, 1991). The hydrophobicity plot displayed seven hydrophobic, putative membrane spanning regions which makes the rat hypothalamic Y5 receptor a member of the G-protein coupled superfamily. The nucleotide and deduced amino acid sequences are shown in Figures 3 and 4, respectively. Like most G-protein coupled receptors, the Y5 receptor contains consensus sequences for N-linked glycosylation, in the amino terminus (position 21 and 28) involved in the proper expression of membrane proteins (Kornfeld and Kornfeld, 1985). The Y5 receptor carries two highly conserved cysteine residues in the first two extracellular loops that are believed to form a disulfide bond stabilizing the functional protein structure (Probst et al, 1992). The Y5 receptor shows 9 potential phosphorylation sites for protein kinase C in positions 204, 217, 254, 273, 285, 301, 328, 336 and 409 and 2 cAMP- and cGMP-dependent protein kinase phosphorylation sites in positions 298 and 370. It should be noted that 8 of

these 11 potential phosphorylation sites are located in the third intra-cellular loop, two in the second intra-cellular loop, and one in the carboxy terminus of the receptor and could therefore play a role in regulating functional characteristics of the Y5 receptor (Probst et al, 1992). In addition, the rat Y5 receptor carries a leucine zipper motif in its first putative transmembrane domain (Landschulz et al, 1988). A tyrosine kinase phosphorylation site is found in the middle of the leucine zipper.

Localization studies (see below) show that the Y5 mRNA is present in several areas of the rat hippocampus. Assuming a comparable localization in human brain, a human hippocampal cDNA library was screened with rat oligonucleotide primers which were shown to yield a DNA band of the expected size in a PCR reaction run on human hippocampal cDNA (C. Gerald, unpublished results). Using this PCR screening strategy (Gerald, Adham, Kao, et al., 1995), three positive pools were identified. One of these pools was analyzed further, and an isolated clone was purified by sib selection. The isolated clone (CG-19) turned out to contain a full length cDNA cloned in the correct orientation for functional expression (see below). The human Y5 nucleotide and deduced amino acid sequences are shown in Figures 5 and 6, respectively. The longest open reading frame encodes a 455 amino acid protein. When compared to the rat Y5 receptor the human sequence shows 84.1% nucleotide identity (Fig. 7A to 7E) and 87.2% amino acid identity (Fig. 7F and 7G). The rat protein sequence is one amino acid longer at the very end of both amino and carboxy tails of the receptor when compared to the human protein sequence. The human 5-6 loop is one amino acid longer than the rat and shows multiple non conservative substitutions. Even though the 5-6 loops show significant changes between the rat and human homologs, all of the protein motifs found in the

rat receptor are present in the human homolog. All putative transmembrane domains and extra cellular loop regions are highly conserved (Fig. 7F and 7G). Therefore, both pharmacological profiles and functional characteristics of the rat and human Y5 receptor subtype homologs may be expected to match closely.

When the human and rat Y5 receptor sequences were compared to other NPY receptor subtypes or to other human G protein-coupled receptor subtypes, both overall and transmembrane domain identities were very low, showing that the Y5 receptor genes are not closely related to any other previously characterized cDNAs (Table 4). Even among the human NPY receptor family, Y1, Y2, Y4 and Y5 members show unusually low levels of amino acid identity (Fig. 8A through 8C).

TABLE 4: Human Y5 transmembrane domains identity with other human NPY receptor subtypes and other human G-protein coupled receptors

Receptor subtype	% TM identity
Y-4	40
Y-2	42
Y-1	42
MUSGIR	32
DroNPY	31
Beta-1	30
Endothelin-1	30
Dopamine D2	29
Adenosine A2b	28
Subst K	28
Alpha-2A	27
5-HT1Dalpha	26
Alpha-1A	26
IL-8	26
5-HT2	25
Subst P	24

It was also discovered, by PCR using Y5-specific primers, that the human neuroblastoma cell line SK-N-MC contains Y5 receptor mRNA, but Y5-specific binding and functional

assays (using agonists) with the cell line were negative. However, a cDNA encoding a functional Y5 receptor was isolated by PCR from the SK-N-MC cell line.

Northern blot analysis

Using the rat Y5 probe, northern hybridizations reveal a strong signal at 2.7 kb and a weak band at 8 kb in rat whole brain. A weak signal is observed at 2.7 kb in testis. No signal was seen in heart, spleen, lung, liver, skeletal muscle and kidney after a three day exposure (Figure 16A). This is in agreement with the 2.7 kb cDNA isolated by expression cloning from rat hypothalamus and indicates that the disclosed cDNA clone is full length. The 8 kb band seen in whole brain probably corresponds to unspliced pre-mRNA.

With the human Y5 probe, northern hybridizations (Figures 16B and 16C) showed a strong signal at 3.5 kb with a much weaker band at 2.2 and 1.1 kb in caudate nucleus, putamen and cerebral cortex, a medium signal in frontal lobe and amygdala and a weak signal in hippocampus, occipital and temporal lobes, spinal cord, medulla, thalamus, subthalamic nucleus, and substantia nigra. No signal at 3.5 kb was detectable in cerebellum or corpus callosum after a 48 h exposure. It should be noted that Clontech's MTN II and III blots do not carry any mRNA from hypothalamus, periaqueductal gray, superior colliculus and raphe.

Southern blot analysis on human genomic DNA reveals a single band pattern in 4 of the 5 restriction digests (Figure 17A). The two bands observed in the PstI digest can be explained by the presence of a PstI site in the coding region of the human Y5 gene. Rat southern blotting analysis showed a single band pattern in all five restriction digests tested (Figure 17B). These

analyses are consistent with the human and rat genomes containing a single copy of the Y5 receptor gene.

Canine Y5 homolog

The longest open reading frame in the canine (beagle) Y5 cDNA (BO11) encodes a 456 amino acid protein with an estimated molecular weight of 50 kD. The full-length deduced canine Y5 amino acid sequence is shown in Figure 24. The canine Y5 receptor is the same length as the rat Y5 receptor, and is one amino acid longer than the human Y5 receptor. The canine Y5 receptor has 94.3% amino acid identity and 91.7% nucleotide identity with the human Y5 receptor. The canine Y5 receptor has 91.6% amino acid identity and 82.8% nucleotide identity with the rat Y5 receptor. Evidence was found for a potential allelic variation in the beagle Y5 receptor. In clones BO11 and BO12 there is a T in position 477, while in clone BO10 and two partial cDNAs, Bgldog5 and Bgldog6, there is a C in this position. Either nucleotide at this position results in an asparagine. Given the high degree of sequence identity among the three species homologues, the pharmacological profile of the canine Y5 receptor subtype is expected to closely resemble the human and rat Y5 profiles.

Binding Studies

The cDNA for the rat hypothalamic Y5 receptor was transiently expressed in COS-7 cells for full pharmacological evaluation. ^{125}I-PYY bound specifically to membranes from COS-7 cells transiently transfected with the rat Y5 receptor construct. The time course of specific binding was measured in the presence of 0.08 nM ^{125}I-PYY at 30°C (Fig. 9). The association curve was monophasic, with an observed association rate (K_{obs}) of 0.06 min^{-1} and a $t_{1/2}$ of 11 min; equilibrium binding was 99% complete within 71 min and stable for at least 180 min. All subsequent binding assays were carried out for

120 min at 30°C. The binding of ^{125}I-PYY to transiently expressed rat Y5 receptors was saturable over a radioligand concentration range of 0.4 pM to 2.7 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.29 nM ($pK_d = 9.54 \pm 0.13$, n = 4). A receptor density of between 5 and 10 pmol/mg membrane protein was measured on membranes which had been frozen and stored in liquid nitrogen (Fig. 10). Membranes from mock-transfected cells, when prepared and analyzed in the same way as those from CG-18-transfected cells, displayed no specific binding of ^{125}I-PYY (data not shown). Applicants conclude that the ^{125}I-PYY binding sites observed under the described conditions were derived from the rat Y5 receptor construct.

A closely related peptide analog, porcine ^{125}I-[Leu^{31},Pro^{34}]PYY, also bound specifically to membranes from COS-7 cells transiently transfected with rat Y5 receptor cDNA. The time course of specific binding was measured at room temperature in both standard binding buffer ([Na^+] = 10 mM) and isotonic binding buffer ([Na^+] = 138 mM) using 0.08 nM ^{125}I-[Leu^{31},Pro^{34}]PYY (Figure 18). The association curve in 10 mM [Na^+] was monophasic, with an observed association rate (K_{obs}) of 0.042 min^{-1} and a $t_{1/2}$ of 17 min; equilibrium binding was 99% complete within 110 min and stable for at least 210 min (specific binding was maximal at 480 fmol/mg membrane protein). The association curve in 138 mM [Na^+] was also monophasic with a slightly slower time course: (K_{obs}) of 0.029 min^{-1} and a $t_{1/2}$ of 24 min.; equilibrium binding was 99% complete within 160 min. and stable for at least 210 min. (specific binding was maximal at 330 fmol/mg membrane protein). Note that the specific binding was reduced as [Na^+] was increased; a similar phenomenon has been observed for other G protein coupled receptors and may reflect a general property of this receptor family to be modulated by Na^+ (Horstman et. al., 1990). Saturation

binding studies were performed with ^{125}I-[Leu^{31},Pro^{34}]PYY in isotonic buffer at room temperature over a 120 minute period. Specific binding to transiently expressed rat Y5 receptors was saturable over a radioligand concentration range of 0.6 pM to 1.9 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.072 nM (pKd = 10.14 + 0.07, n = 2). A receptor density of 560 $\pm$ 150 pmol/mg on membranes which had been frozen and stored in liquid nitrogen. That ^{125}I-[Leu^{31},Pro^{34}]PYY can bind to the rat Y5 receptor with high affinity at room temperature in isotonic buffer makes it a potentially useful ligand for characterizing the native Y5 receptor in rat tissues using autoradiographic techniques. Care must be taken, however, to use appropriate masking agents to block potential radiolabeling of other receptors such as Y1 and Y4 receptors (note in Table 6 that rat Y1 and Y4 bind the structural homolog [Pro^{34}]PYY). Previously published reports of ^{125}I-[Leu^{31},Pro^{34}]PYY as a Y1-selective radioligand should be re-evaluated in light of new data obtained with the rat Y5 receptor (Dumont et al., 1995).

The pharmacological profile of the rat Y5 receptor was first studied by using pancreatic polypeptide analogs in membrane binding assays. The rank order of affinity for selected compounds was derived from competitive displacement of ^{125}I-PYY (Fig. 11). The rat Y5 receptor was compared with cloned Y1, Y2, and Y4 receptors from human (Table 5) and rat (Table 6), all expressed transiently in COS-7 cells. One receptor subtype absent from our panel was the Y3, human or rat, as no model suitable for radioligand screening has yet been identified.

TABLE 5: Pharmacological profile of the rat Y5 receptor vs. Y-type receptors cloned from human.

Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing rat

Y5 and human subtype clones. Peptides were tested at concentrations ranging from 0.001 nM to 1000 nM unless noted. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the Cheng-Prusoff equation. The data shown are representative of at least two independent experiments.

TABLE 5

Peptide	K_i Values (nM)			
	Rat Y5	Human Y4	Human Y1	Human Y2
rat/human NPY	**0.68**	2.2	0.07	0.74
porcine NPY	**0.66**	1.1	0.05	0.81
human NPY_{2-36}	**0.86**	16	3.9	2.0
porcine NPY_{2-36}	**1.2**	5.6	2.4	1.2
porcine NPY_{13-36}	**73**	38	60	2.5
porcine NPY_{26-36}	**> 1000**	304	> 1000	380
porcine C2-NPY	**470**	120	79	3.5
human $[Leu^{31}, Pro^{34}]$NPY	**1.0**	1.1	0.17	> 130
human $[D-Trp^{32}]$NPY	**53**	> 760	> 1000	> 1000
human NPY free acid	**480**	> 1000	490	> 1000
rat/porcine PYY	**0.64**	0.14	0.35	1.26
human PYY	**0.87**	0.87	0.18	0.36

human PYY_{3-36}	**8.4**	15	41	0.70
human PYY_{13-36}	**190**	46	33	1.5
human $[Pro^{34}]PYY$	**0.52**	0.12	0.14	> 310
human PP	**5.0**	0.06	77	> 1000
human PP_{2-36}*	**not tested**	0.06	> 40	> 100
human PP_{13-36}*	**not tested**	39	> 100	> 100
rat PP	**180**	0.16	450	> 1000
salmon PP	**0.31**	3.2	0.11	0.17

*Tested only up to 100 nM.

TABLE 6: Pharmacological profile of the rat Y5 receptor vs. Y-type receptors cloned from rat.

Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing rat Y5 and rat subtype clones. Peptides were tested at concentrations ranging from 0.001 nM to 1000 nM. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the Cheng-Prusoff equation. The data shown are representative of at least two independent experiments. Exception: new peptides (marked with a double asterisk) were tested in one or more independent experiments.

TABLE 6

Peptide	K_i Values (nM)			
	Rat Y5	Rat Y4	Rat Y1	Rat Y2
rat/human NPY	**0.68**	1.7	0.12	1.3
porcine NPY **	**0.66**	1.78	0.06	1.74
frog NPY ** (melanostatin)	**0.71**		0.09	0.65
human NPY_{2-36}	**0.86**	5.0	12	2.6
porcine NPY_{2-36} **	**1.1**	18	1.6	1.6
porcine NPY_{3-36} **	**7.7**	36	91	3.7
porcine NPY_{13-36}	**73**	140	190	31
porcine NPY_{16-36} **	**260**	200	140	35
porcine NPY_{18-36} **	**> 1000**		470	12
porcine NPY_{20-36} **	**> 100**		360	93

Peptide	K_i Values (nM)			
	Rat Y5	Rat Y4	Rat Y1	Rat Y2
porcine NPY_{22-36} **	**> 1000**		> 1000	54
porcine NPY_{26-36} **	**> 1000**		> 1000	> 830
human $[Leu^{31},Pro^{34}]NPY$	**1.0**	0.59	0.10	> 1000
porcine ** $[Leu^{31},Pro^{34}]NPY$	**1.6**	0.32	0.25	840
human (O-Methyl-Tyr^{21})NPY **	**1.6**			2.3
human NPY free acid **	**> 610**	> 1000	720	> 980
porcine C2-NPY **	**> 260**	22	140	2.6
human NPY_{1-24} amide **	**> 1000**		> 320	> 1000
human [D-Trp^{32}]NPY	**35**	> 630	> 1000	760
rat/porcine PYY	**0.64**	0.58	0.21	0.28
human PYY **	**0.87**		0.12	0.30
human PYY_{3-36} **	**8.4**	15		0.48
human PYY_{13-36} **	**290**		130	14
human $[Pro^{34}]PYY$	**0.52**	0.19	0.25	> 1000
porcine $[Pro^{34}]PYY$ **	**0.64**	0.24	0.07	> 980
avian PP **	**> 930**	> 81	> 320	> 1000

Table 6 continued

Peptide	K_i Values (nM)			
	Rat Y5	Rat Y4	Rat Y1	Rat Y2
human PP	**5.0**	0.04	43	> 1000
human PP_{13-36} **	**84**		> 1000	> 650
human PP_{31-36} **	**> 1000**	26	> 10 000	> 10 000
human PP_{31-36} free acid **	**>10,000**	> 100		
bovine PP **	**8.4**	0.19	120	> 1000
frog PP (rana temporaria) **	**> 550**	> 1000	720	> 980
rat PP	**230**	0.19	350	> 1000
salmon PP	**0.33**	3.0	0.30	0.16
PYX-1 **	**920**			
PYX-2 **	**> 1000**			
FLRF-amide **	**5500**		45 000	
FMRF-amide **	**18000**			
W(nor-L)RF-amide **	**8700**			

The rat Y5 receptor possessed a unique pharmacological profile when compared with human and rat Y-type receptors. It displayed a preference for structural analogs of rat/human NPY (K_i = 0.68 nM) and rat/porcine PYY (K_i = 0.64 nM) over most PP derivatives. The high affinity for salmon PP (K_i = 0.31 nM) reflects the close similarity between salmon PP and rat NPY, sharing 81% of their amino acid sequence and maintaining identity at key positions: Tyr^1, Gln^{34}, and Tyr^{36}. Both N- and C-terminal peptide domains are apparently important for receptor recognition. The N-terminal tyrosine of NPY or PYY could be deleted without an appreciable loss in binding

affinity (K_i = 0.86 nM for rat/human NPY_{2-36}), but further N-terminal deletion was disruptive (K_i = 73 nM for porcine NPY_{13-36}). A similar structure-activity relationship was observed for PYY and N-terminally deleted fragments such as PYY_{3-36} and PYY_{13-36}. This pattern places the binding profile of the Y5 receptor somewhere between that of the Y2 receptor (which receptor can withstand extreme N-terminal deletion) and that of the Y1 receptor (which receptor is sensitive to even a single-residue N-terminal deletion). Note that the human Y4 receptor can be described similarly (K_i = 0.06 nM for human PP, 0.06 nM for human PP_{2-36}, and 39 nM for human PP_{13-36}). The Y5 receptor resembled both Y1 and Y4 receptors in its tolerance for ligands containing Pro^{34} (as in human $[Leu^{31},Pro^{34}]$NPY, human $[Pro^{34}]$-PYY, and human PP). Interestingly, the rat Y5 receptor displayed a preference for human PP (K_i = 5.0 nM) over rat PP (K_i = 180 nM). This pattern distinguishes the rat Y5 from the rat Y4 receptor, which binds both human and rat PP with K_i values $<$ 0.2 nM. Hydrolysis of the carboxy terminal amide to free carboxylic acid, as in NPY free acid, was disruptive for binding affinity for the rat Y5 receptor (K_i = 480 nM). The terminal amide appears to be a common structural requirement for pancreatic polypeptide family/receptor interactions.

Several peptides shown previously to stimulate feeding behavior in rats bound to the rat Y5 receptor with $K_i \leq$ 5.0 nM. These include rat/human NPY (K_i = 0.68 nM), rat/porcine PYY (K_i = 0.64 nM), rat/human NPY_{2-36} (K_i = 0.86 nM), rat/human $[Leu^{31},Pro^{34}]$NPY (K_i = 1.0 nM), and human PP (K_i = 5.0 nM). Conversely, peptides which were relatively less effective as orexigenic agents bound weakly to CG-18. These include porcine NPY_{13-36} (K_i = 73 nM), porcine C2-NPY (K_i = 470 nM) and human NPY free acid (K_i = 480 nM). The rank order of K_i values are in agreement with rank orders of potency and activity for

stimulation of feeding behavior when peptides are injected i.c.v. or directly into rat hypothalamus (Clark et al., 1984; Stanley et al., 1985; Kalra et al., 1991; Stanley et al., 1992). The rat Y5 receptor also displayed moderate binding affinity for [D-Trp^{32}]NPY (K_i = 53 nM), the modified peptide reported to regulate NPY-induced feeding by Balasubramaniam et al. (1994). It is noteworthy that [D-Trp^{32}]NPY was ≥ 10-fold selective for CG-18 over the other cloned receptors studied, whether human or rat. These data clearly and definitively link the cloned Y5 receptor to the feeding response.

The cDNA corresponding to the human Y5 homolog isolated from human hippocampus was transiently expressed in COS-7 cells for membrane binding studies. The binding of ^{125}I-PYY to the human Y5 receptor (CG-19) was saturable over a radioligand concentration range of 8 pM to 1.8 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.10 nM in the first experiment. Repeated testing yielded an apparent K_d of 0.18 nM (pK_d = 9.76 ± 0.11, n = 4). A maximum receptor density of 500 fmol/mg membrane protein was measured on fresh membranes. As determined by using peptide analogs within the pancreatic polypeptide family, the human Y5 pharmacological profile bears a striking resemblance to the rat Y5 receptor (Tables 7 and 8).

TABLE 7: Pharmacological profile of the rat Y5 receptor vs. the human Y5 receptor, as expressed both transiently in COS-7 and stably in LM(tk-) cells.

Binding data reflect competitive displacement of radioligand (either ^{125}I-PYY or ^{125}I-PYY$_{3-36}$ as indicated) from membranes of COS-7 cells transiently expressing the rat Y5 receptor and its human homolog or from LM(tk-) cells stably expressing the human Y5 receptor. Peptides were tested at concentrations ranging from 0.001 nM to 1000 nM. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the Cheng-Prusoff equation. New peptides are marked with a double asterisk.

TABLE 7

Peptide	K_i Values (nM)			
	Rat Y5 (COS-7, ^{125}I-PYY)	Human Y5 (COS-7, ^{125}I-PYY)	Human Y5 (LM(tk-), ^{125}I-PYY)	Human Y5 (LM(tk-), ^{125}I-PYY$_{3-36}$)
rat/human NPY	0.68	0.15	0.89	0.65
porcine NPY **		0.68	1.4	
human NPY$_{2-36}$	0.86	0.33	1.6	0.51
porcine NPY$_{2-36}$ **	0.66	0.58	1.2	
porcine NPY$_{13-36}$	73	110		39
porcine NPY$_{16-36}$ **	260	300		180
porcine NPY$_{18-36}$ **	> 1000	> 470		310

Peptide	K_i Values (nM)			
	Rat Y5 (COS-7, ^{125}I-PYY)	Human Y5 (COS-7, ^{125}I-PYY)	Human Y5 (LM(tk-), ^{125}I-PYY)	Human Y5 (LM(tk-), ^{125}I-PYY_{3-36})
porcine NPY_{22-36} **	> 1000	> 1000		
porcine NPY_{26-36} **	> 1000	> 1000		
human [Leu^{31}, Pro^{34}] NPY	1.0	0.72	3.0	
human [Leu^{31}, Pro^{34}] NPY **			2.4	1.4
human NPY free acid **	> 610	> 840		
porcine C2-NPY **	260	370	260	220
human [D-Trp^{32}]NPY	35	35	16	10
rat/porcine PYY	0.64	0.75		
human PYY **	0.87	0.44	1.3	0.43
human PYY_{3-36} **	8.4	17	8.1	1.6
human [Pro^{34}]PYY	0.52	0.34	1.7	1.7
human PP	5.0	1.7	3.0	1.2
human PP_{2-36} **		2.1		
human PP_{13-36} **	290	720		

Table 7 continued

Peptide	K_i Values (nM)			
	Rat Y5 (COS-7, ^{125}I-PYY)	Human Y5 (COS-7, ^{125}I-PYY)	Human Y5 (LM(tk-), ^{125}I-PYY)	Human Y5 (LM(tk-), ^{125}I-PYY_{3-36})
human PP_{31-36} **	> 10 000	> 10 000		41 000
human [Ile^{31},Gln^{34}] PP **		2.0		
bovine PP **	8.4	1.6	7.9	5.0
rat PP	230	630		130
salmon PP	0.33	0.27		0.63

TABLE 8: Pharmacological profile of the human Y5 receptor vs. Y-type receptors cloned from human.

Binding data reflect competitive displacement of ^{125}I-PYY from membranes of COS-7 cells transiently expressing human Y5 other sub-type clones. Peptides were tested at concentrations ranging from 0.001 nM to 1000 nM unless noted. IC_{50} values corresponding to 50% displacement were determined by nonlinear regression analysis and converted to K_i values according to the Cheng-Prusoff equation. The data shown are representative of at least two independent experiments.

TABLE 8

Peptide	K_i Values (nM)			
	Human Y5	Human Y4	Human Y1	Human Y2
rat/human NPY	**0.46**	2.2	0.07	0.74
porcine NPY	**0.68**	1.1	0.05	0.81
human NPY_{2-36}	**0.75**	16	3.9	2.0
porcine NPY_{2-36}	**0.58**	5.6	2.4	1.2
porcine NPY_{13-36}	**110**	38	60	2.5
porcine NPY_{26-36}	**> 1000**	304	> 1000	380
porcine C2-NPY	**370**	120	79	3.5
human [Leu^{31},Pro^{34}]NPY	**1.6**	1.1	0.17	> 130
human [D-Trp^{32}]NPY	**35**	> 760	> 1000	> 1000
human NPY free acid	**> 840**	> 1000	490	> 1000
rat/porcine PYY	**0.58**	0.14	0.35	1.26
human PYY	**0.44**	0.87	0.18	0.36
human PYY_{3-36}	**17**	15	41	0.70

Peptide	K_i Values (nM)			
	Human Y5	Human Y4	Human Y1	Human Y2
human PYY_{13-36}	**not tested**	46	33	1.5
human $[Pro^{34}]PYY$	**0.77**	0.12	0.14	> 310
human PP	**1.4**	0.06	77	> 1000
human PP_{2-36}*	**2.1**	0.06	> 40	> 100
human PP_{13-36}*	**720**	39	> 100	> 100
rat PP	**630**	0.16	450	> 1000
salmon PP	**0.46**	3.2	0.11	0.17

*Tested only up to 100 nM.

Binding Studies of hY5 Expressed in Insect Cells

Tests were initially performed to optimize expression of hY5 receptor. Infecting Sf9, Sf21, and High Five cells with hY5BB3 virus at a multiplicity of infection (MOI) of 5 and preparing membranes for binding analyses at 45 hrs. postinfection, B_{max} ranges from 417 to 820 fmoles/mg protein, with the highest expression being hY5BB3 in Sf21 cells were observed. Therefore, the next series of experiments used Sf21 cells. Optimal multiplicity of infection (the ratio of viral particles to cells) was next examined by testing MOI of 1, 2, 5 and 10. The B_{max} values were ≈1.1-1.2 pmoles/mg protein for any of the MOIs, suggesting that increasing the number of viral particles per cell is neither deleterious nor advantageous. Since viral titer calculations are approximate, MOI=5 was used for future experiments. The last parameter tested was hours postinfection for protein expression, ranging from 45-96 hours postinfection. It was found that optimal expression occurred 45-73 hrs. postinfection. In summary, a hY5 recombinant baculovirus has been created which binds ^{125}I-PYY with a B_{max} of ≈1.2 pmoles/mg protein.

Human Y5 Homolog: Transient Expression in Baculovirus-Infected Sf21 Insect Ovary Cells

Sf21 cells infected with a human Y5 baculovirus construct were harvested as membrane homogenates and screened for specific binding of ^{125}I-PYY using 0.08 nM radioligand. Specific binding was greatest (500 fmol/mg membrane protein) for sample D-2/[4], derived from Sf-21 cells. No specific binding was observed after infection with the baculovirus plasmid alone (data not shown). If the assumption is made that the binding affinity of porcine ^{125}I-PYY for the human Y5 receptor is the same whether the expression system is COS-7 or baculovirus/Sf-21 (0.18 nM), the specific binding in sample D-2/[4] predicts an apparent B_{max} of 1600 fmol/mg membrane protein. The Y5

receptor yield in the baculovirus/Sf21 expression system is therefore as good or better than that in COS-7. We conclude that the baculovirus offers an alternative transfection technique amenable to large batch production of the human Y5 receptor.

Binding Studies Using the Canine Y5 Receptor

Membranes from COS-7 cells transiently transfected with canine Y5 receptor (using the plasmid designated cY5-BO11, ATCC Accession No. 97587) displayed specific binding of porcine ^{125}I-PYY. The binding was saturable over a concentration range of 0.6 pM to 2.7 nM, with an observed K_d of 1.1 nM and a B_{max} of 5700 fmol/mg membrane protein. Compounds selected for the ability to bind or activate the human and rat Y5 receptor homologs were subsequently tested for binding to the canine Y5 receptor (Table 20). The pharmacological profile for the canine Y5 receptor was in general agreement with those derived for the other species homologs. For example, the canine Y5 receptor bound human NPY, PYY and PP with K_i values < 10 nM. The canine Y5 receptor bound bovine PP with higher affinity (10 nM) than rat PP (160 nM), as is also the case for the rat and human Y5 receptor homologs. Binding affinity was not disturbed by substitution of Gln^{34} in NPY or PYY with Pro^{34} (as in $[Leu^{31},Pro^{34}]$NPY, K_i = 4.1 or $[Pro^{34}]$PYY, K_i = 1.4 nM). In this regard, the canine Y5 receptor exhibits what has been historically perceived as a Y1-like property. It was also observed that deletion of Tyr^1 from NPY (as in NPY_{2-36}) was not disruptive (K_i = 2.1 nM). Further deletion of NPY and PYY to fragments such as NPY_{3-36}, PYY_{3-36} and NPY_{13-36}, however, was increasingly disruptive. The canine Y5 receptor bound the Y2-selective and centrally modified analog C2-NPY with relatively weak affinity (K_i = 300 nM). It is concluded that the canine Y5 receptor, like the rat and human Y5 counterparts, depends on selected residues in the N-terminal, central and C-terminal regions of the

parent peptide for optimal binding affinity. Particularly diagnostic tools such as the Y5-selective peptide D-[Trp^{32}]NPY and the Y1-selective antagonist BIBP 3226 (Rudolf, et al., 1994) were bound by the canine Y5 receptor with K_i values of 35 and 17000 nM, respectively. These values are in the range of those reported for the rat and human Y5 homologs.

BIBP 3226 was also tested for binding affinity at the cloned human Y-type receptors, and was observed to bind with K_i values of 14 nM for the Y1 receptor, 6900 nM for the Y2 receptor, 8000 nM for the Y4 receptor and 49000 nM for the Y5 receptor. Similar experiments with cloned rat Y-type receptors generated K_i values of 20 nM for the Y1 receptor, 66000 nM for the Y2 receptor, 420 nM for the Y4 receptor and 25000 nM for the Y5 receptor. BIBP 3226 blocked NPY-induced activation of rat Y1 receptors with a K_b of 9.4 nM and also blocked PP-induced activation of rat Y4 receptors with a Kb of 4800 uM; there was no evidence for antagonism of NPY- or PP-induced activation of rat Y2 or Y5 receptors at concentrations up to 1 μM. These data further confirm the classification of BIBP 3226 as a Y1-selective receptor antagonist.

<u>Stable Expression Systems for Y5 Receptors: Characterization in Binding Assays</u>

The cDNA for the rat Y5 receptor was stably transfected into 293 cells which were pre-screened for the absence of specific ^{125}I-PYY binding (data not shown). After co-transfection with the rat Y5 cDNA plus a G-418-resistance gene and selection with G-418, surviving colonies were screened as membrane homogenates for specific binding of ^{125}I-PYY using 0.08 nM radioligand. A selected clone (293 clone # 12) bound 65 fmol ^{125}I-PYY /mg membrane protein and was isolated for further study in functional assays.

The cDNA for the human Y5 receptor was stably transfected

into both NIH-3T3 and LM(tk-) cells, each of which were pre-screened for the absence of specific ^{125}I-PYY binding (data not shown). After co-transfection with the human Y5 cDNA plus a G-418-resistance gene and selection with G-418, surviving colonies were screened as membrane homogenates for specific binding of ^{125}I-PYY using 0.08 nM radioligand. NIH-3T3 clone #8 bound 46 fmol ^{125}I-PYY/mg membrane protein and LM(tk-) clone #7 bound 32 fmol ^{125}I-PYY/mg membrane protein. These two clones were isolated for further characterization in binding and cAMP functional assays. A third clone which bound 25 fmol/mg membrane protein, LM(tk-) #3, was evaluated in calcium mobilization assays.

The human Y5 stably expressed in NIH-3T3 cells (clone #8) was further characterized in saturation binding assays using ^{125}I-PYY. The binding was saturable over a concentration range of 0.4 pM to 1.9 nM. Binding data were fit to a one-site binding model with an apparent K_d of 0.30 nM (pK_d = 9.53, n = 1) and an apparent B_{max} of 2100 fmol/mg membrane protein using fresh membranes.

The human Y5 stably expressed in LM(tk-) cells (clone #7) was further characterized in saturation binding assays using ^{125}I-PYY, ^{125}I-PYY$_{3-36}$, and ^{125}I-NPY. ^{125}I-PYY binding was saturable according to a 1-site model over a concentration range of 0.4 pM to 1.9 nM, with an apparent K_d of 0.47 nM (pK_d = 9.32 $\pm$ 0.07, n = 5) and an apparent B_{max} of up to 8 pmol/mg membrane protein when membranes had been frozen and stored in liquid nitrogen. Peptide K_i values derived from ^{125}I-PYY binding to human Y5 receptors from LM(tk-) were comparable to those derived from the previously described human and rat Y5 expression systems (Table 7). ^{125}I-PYY$_{3-36}$ binding to the human Y5 in LM(tk-) cells was also saturable according to a 1-site model over a concentration range of 0.5 pM to 2.09 nM, with an apparent K_d of 0.40 nM (pK_d = 9.40, n = 1) and an apparent

B_{max} of 490 fmol/mg membrane protein when membranes had been frozen and stored in liquid nitrogen. Peptide ligands appeared to bind with comparable affinity to human Y5 receptors in LM(tk-) cells whether the radioligand used was ^{125}I-PYY or ^{125}I-PYY$_{3-36}$ (Table 7). Finally, ^{125}I-NPY binding to the human Y5 in LM(tk-) cells was saturable according to a 1-site model over a concentration range of 0.4 pM to 1.19 nM, with an apparent K_d of 0.28 and an apparent B_{max} of 360 fmol/mg membrane protein when membranes had been frozen and stored in liquid nitrogen.

Considering the saturation binding studies for the human and rat Y5 receptor homologs as a whole, the data provide evidence that the Y5 receptor is a target for multiple radioiodinated peptide analogs in the pancreatic polypeptide family, including ^{125}I-PYY, ^{125}I-NPY, ^{125}I-PYY$_{3-36}$, and ^{125}I-[Leu^{31},Pro^{34}]PYY. The so-called Y1 and Y2-selective radioligands (such as ^{125}I-[Leu^{31},Pro^{34}]PYY and ^{125}I-PYY$_{3-36}$, respectively (Dumont et al., 1995)) should be used with caution when probing native tissues for Y-type receptor expression.

Receptor/G protein Interactions: Effects of Guanine Nucleotides

For a given G protein-coupled receptor, a portion of the receptor population can typically be characterized in the high affinity ligand binding site using discriminating agonists. The binding of GTP or a non-hydrolyzable analog to the G protein causes a conformational change in the receptor which favors a low affinity ligand binding state. Whether the non-hydrolyzable GTP analog, Gpp(NH)p, would alter the binding of ^{125}I-PYY to Y5 in COS-7 and LM(tk-) cells (Fig 19) was investigated. ^{125}I-PYY binding to both human and rat Y5 receptors in COS-7 cells was relatively insensitive to increasing concentrations of Gpp(NH)p ranging from 1 nM to 100 μM (Fig. 19), as was

also the case for dog Y5 receptors in COS-7 cells (data not shown). The human Y5 receptor in LM(tk-) cells, however, displayed a concentration dependent decrease in radioligand binding (~85 fmol/mg membrane protein over the entire concentration range). The difference between the receptor preparations could be explained by several factors, including 1) the types of G proteins available in the host cell for supporting a high affinity receptor-agonist complex, 2) the level of receptor reserve in the host cell, 3) the efficiency of receptor/G protein coupling, and 4) the intrinsic ability of the agonist (in this case, ^{125}I-PYY) to distinguish between multiple conformations of the receptor.

Functional Assay

Activation of all Y-type receptors described thus far is thought to involve coupling to pertussis toxin-sensitive G-proteins which are inhibitory for adenylate cyclase activity (G_i or G_o) (Wahlestedt and Reis, 1993). That the atypical Y1 receptor is linked to cyclase inhibition was prompted by the observation that pertussis toxin inhibited NPY-induced feeding in vivo (Chance et al., 1989); a more definitive analysis was impossible in the absence of the isolated receptor. Based on these prior observations, the ability of NPY to inhibit forskolin-stimulated cAMP accumulation in human embryonic kidney 293 cells stably transfected with rat Y5 receptors was investigated. Incubation of intact cells with 10 μM forskolin produced a 10-fold increase in cAMP accumulation over a 5 minute period, as determined by radioimmunoassay. Simultaneous incubation with rat/human NPY decreased the forskolin-stimulated cAMP accumulation by 67% in stably transfected cells (Fig. 12), but not in untransfected cells (data not shown). It is concluded that the rat Y5 receptor activation results in decreased cAMP accumulation, very likely through inhibition of adenylate cyclase activity. This result is consistent

with the proposed signalling pathway for all Y-type receptors and for the atypical Y1 receptor in particular.

Peptides selected for their ability to stimulate feeding behavior in rats were able to activate the rat Y5 receptor with EC_{50} < 10 nM (Kalra et al., 1991; Stanley et al., 1992; Balasubramaniam et al., 1994). These include rat/human NPY (EC_{50} = 1.8 nM), rat/human NPY_{2-36} (EC_{50} = 2.0 nM), rat/human [Leu^{31},Pro^{34}]NPY (EC_{50} = 0.6 nM), rat/porcine PYY (EC_{50} = 4.0 nM), and rat/human [D-Trp^{32}]NPY (EC_{50} = 7.5 nM) (Table 9). K_i values derived from rat Y5-dependent binding of ^{125}I-PYY and peptide ligands (Table 5) were in close range of EC_{50} values derived from rat Y5-dependent regulation of cAMP accumulation (Table 9). The maximal suppression of cAMP produced by all peptides in Table 9 was between 84% and 120% of that produced by human NPY, except in the case of FLRFamide (42%). Of particular interest is the Y5-selective peptide [D-Trp^{32}]NPY. This is a peptide which was shown to stimulate food intake when injected into rat hypothalamus, and which also attenuated NPY-induced feeding in the same paradigm (Balasubramaniam, 1994). It was observed that [D-Trp^{32}]NPY bound weakly to other Y-type clones with K_i > 500 nM (Tables 5 and 6) and displayed no activity in functional assays (Table 11). In striking contrast, [D-Trp^{32}]NPY bound to the rat Y5 receptor with a K_i = 53 nM and was fully able to mimic the inhibitory effect of NPY on forskolin-stimulated cAMP accumulation with an EC_{50} of 25nm and an E_{max} = 72%. That [D-Trp^{32}]NPY was able to selectively activate the Y5 receptor while having no detectable activity at the other subtype clones strongly suggests that Y5 receptor activation is responsible for the stimulatory effect of [D-Trp^{32}]NPY on feeding behavior in vivo.

TABLE 9: Functional activation of the rat Y5 receptor. Functional data were derived from radioimmunoassay of

cAMP accumulation in stably transfected **293** cells stimulated with 10 μM forskolin. Peptides were tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 μM. The maximum inhibition of cAMP accumulation (E_{max}) and the concentration producing a half-maximal effect (EC_{50}) were determined by nonlinear regression analysis according to a 4 parameter logistic equation. New peptides are marked with a double asterisk.

TABLE 9

Peptide	E_{max}	EC_{50} (nM)
rat/human NPY	67 %	1.8
porcine NPY **		0.79
rat/human NPY_{2-36}	84 %	2.0
porcine NPY_{2-36} **		1.2
porcine NPY_{13-36} **		21
rat/human [Leu^{31},Pro^{34}]NPY	70 %	0.6
porcine [Leu^{31},Pro^{34}]NPY **		1.1
porcine C2-NPY **		240

Table 9 continued

Peptide	E_{max}	EC_{50} (nM)
rat/human [D-Trp^{32}]NPY	72 %	9.5
rat/porcine PYY	86 %	4.0
human PYY **		1.5
human PYY_{3-36} **		4.9
human [Pro^{34}]PYY **		1.8
human PP **		1.4
bovine PP **		5.7
salmon PP **		0.92
rat PP **		130
PYX-1 **		> 300
PYX-2 **		> 300
FLRFamide **		13 000

The ability of the human Y5 receptor to inhibit cAMP accumulation was evaluated in NIH-3T3 and LM(tk-) cells, neither of which display an NPY-dependent regulation of [cAMP] without the Y5 construct. Intact cells stably transfected with the human Y5 receptor were analyzed as described above for the rat Y5 cAMP assay. Incubation of stably transfected NIH-3T3 cells with 10 uM forskolin generated an average 21-fold increase in [cAMP] (n = 2).

Simultaneous incubation with human NPY decreased the forskolin-stimulated [cAMP] with an E_{max} of 42% and an EC_{50} of 8.5 nM (Fig 20). The technique of suspending and then replating the Y5-transfected LM(tk-) cells was correlated with a robust and reliable cellular response to NPY-like peptides and was therefore incorporated into the standard methodology for the functional evaluation of the human Y5 in LM(tk-). Incubation of stably transfected LM(tk-) cells prepared in this manner produced an average 7.4-fold increase in [cAMP] (n = 87). Simultaneous incubation with human NPY decreased the forskolin-stimulated [cAMP] with an E_{max} of 72% and with an EC_{50} of 2.4 nM (Fig 20). The human Y5 receptor supported a cellular response to NPY-like peptides in a rank order similar to that described for the rat Y5 receptor (Table 6, 10). As the rat Y5 receptor is clearly linked by [D-Trp^{32}]NPY and other pharmacological tools to the NPY-dependent regulation of feeding behavior, the human Y5 receptor is predicted to function in a similar fashion. Both the human and receptor homologs represent useful models for the screening of compounds intended to modulate feeding behavior by interfering with NPY-dependent pathways.

TABLE 10: Functional activation of the human Y5 receptor in a cAMP radioimmunoassay.

Functional data were derived from radioimmunoassay of cAMP accumulation in stably transfected LM(tk-) cells stimulated with 10 μM forskolin. Peptides were tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 μM. The maximum inhibition of cAMP accumulation (E_{max}) and the concentration producing a half-maximal effect (EC_{50}) were determined by nonlinear regression analysis according to a 4 parameter logistic equation.

TABLE 10

Peptide	% inhibition relative to human NPY	EC_{50} (nM)
rat/human NPY	100%	2.7
porcine NPY	107%	0.99
rat/human NPY_{2-36}	116%	2.6
porcine NPY_{2-36}	85%	0.71
porcine NPY_{13-36}		49
rat/human $[Leu^{31}, Pro^{34}]NPY$		3.0
porcine $[Leu^{31}, Pro^{34}]NPY$		1.3
rat/human [D-Trp^{32}]NPY	108%	26
rat/porcine PYY	109%	3.6
human PYY	111%	4.9
human PYY_{3-36}		18
human $[Pro^{34}]PYY$	108%	2.5
human PP	96%	14
human PP_{2-36}		2.0
human $[Ile^{31}, Gln^{34}]PP$		5.6
bovine PP		4.0
salmon PP	96%	4.5

TABLE 11: Binding and functional characterization of [D-Trp^{32}]NPY.

Binding data were generated as described in Tables 5 and 6. Functional data were derived from radioimmunoassay of cAMP accumulation in stably transfected cells stimulated with 10 μM forskolin. [D-Trp^{32}]NPY was tested for agonist activity at concentrations ranging from 0.03 pM to 0.3 μM. Alternatively, [D-Trp^{32}]NPY was included as a single spike (0.3 μM) in the human PYY concentration curve for human Y1 and human Y2 receptors, or in the human PP concentration curve for human Y4 receptors, and antagonist activity was detected by the presence of a rightward shift (from EC_{50} to EC_{50}'). K_b values were calculated according to the equation: K_b = [[D-Trp^{32}]NPY/((EC50/EC_{50}')-1). The data shown are representative of at least two independent experiments.

TABLE 11

Receptor Subtype	Species	Binding	Function		
		K_i (nM)	EC_{50} (nM)	K_b (nM)	Activity
Y1	Human	> 1000			None detected
Y2	Human	> 1000			None detected
Y4	Human	> 1000			None detected
Y5	Human	18	**26**		Not Determined
Y1	Rat	> 1000			Not Determined
Y2	Rat	>1000			Not Determined
Y4	Rat	> 1000			Not Determined
Y5	Rat	53	**9.50**		Agonist

Functional Assay: Intracellular Calcium Mobilization

The intracellular free calcium concentration was increased in LM(tk-) cells stably transfected with the human Y5 receptor within 30 seconds of incubation with 100 nM human NPY (Δ Ca^{2+} = 34nM, Fig 21D). Untransfected LM(tk-) cells did not respond to human NPY (data not shown). The calcium mobilization provides a second pathway through which Y5 receptor activation can be measured. These data also serve to link with the Y5 receptor with other cloned human Y-type receptors, all of which have been demonstrated to mobilize intracellular calcium in various expression systems (Fig 21).

Localization Studies

The mRNA for the NPY Y5 receptor was widely distributed in rat brain, and appeared to be moderately abundant (Table 12 and Fig. 13). The midline thalamus contained many neurons with silver grains over them, particularly the paraventricular thalamic nucleus, the rhomboid nucleus, and the nucleus reunions. In addition, moderately intense hybridization signals were observed over neurons in both the centromedial and anterodorsal thalamic nuclei. In the hypothalamus, a moderate level of hybridization signal was seen over scattered neurons in the lateral hypothalamus, paraventricular, supraoptic, arcuate, and dorsomedial nuclei. In both the medial preoptic nucleus and suprachiasmatic nucleus, weak or moderate accumulations of silver grains were present. In the suprachiasmatic nucleus, hybridization signal was restricted mainly to the ventrolateral subdivision. In the paraventricular hypothalamus, positive neurons were observed primarily in the medial parvicellular subdivision.

TABLE 12: Distribution of NPY Y5 mRNA in the Rat CNS

REGION	Y5 mRNA
Cerebral cortex	+1
Thalamus	
paraventricular n.	+3
rhomboid n.	+3
reunions n.	+3
anterodorsal n.	+2
Hypothalamus	
paraventricular n.	+2
lateral hypoth. area	+2 /+3
supraoptic n.	+1
medial preoptic n.	+2
suprachiasmatic n.	+1/+2
arcuate n.	+2
Hippocampus	
dentate gyrus	+1
polymorph dentate gyrus	+2
CA1	0
CA3	+1
Amygdala	
central amygd. n., medial	+2
anterior cortical amygd. n.	+2
Olivary pretectal n.	+3
Anterior pretectal n.	+3
Substantia nigra, pars compacta	+2
Superior colliculus	+2
Central gray	+2
Rostral linear raphe	+3
Dorsal raphe	+1
Inferior colliculus	+1
Medial vestibular n.	+2/+3
Parvicellular ret. n.,alpha	+2
Gigantocellular reticular n., alpha	+2
Pontine nuclei	+1/+2

Moderate hybridization signals were found over most of the neurons in the polymorphic region of the dentate gyrus in the hippocampus, while lower levels were seen over scattered neurons in the CA3 region. In the amygdala, the central nucleus and the anterior cortical nucleus contained neurons with moderate levels of hybridization signal. In the mesencephalon, hybridization signals were observed over a number of areas. The most intense signals were found over neurons in the anterior and olivary pretectal nuclei, periaquaductal gray, and over the rostral linear raphe. Moderate hybridization signals were observed over neurons in the internal gray layer of the superior colliculus, the substantia nigra, pars compacta, the dorsal raphe, and the pontine nuclei. Most of the neurons in the inferior colliculus exhibited a low level of signal. In the medulla and pons, few areas exhibited substantial hybridization signals. The medial vestibular nucleus was moderately labeled, as was the parvicellular reticular nucleus, pars alpha, and the gigantocellular reticular nucleus.

Little or no hybridization signal was observed on sections hybridized with the radiolabeled sense oligonucleotide probe. More importantly, in the transfected COS-7 cells, the antisense probe hybridized only to the cells transfected with the rat Y5 cDNA (Table 13). These results indicate that the probe used to characterize the distribution of Y5 mRNA in rat brain is specific for this mRNA, and does not cross-hybridize to any of the other known NPY receptor mRNAs.

TABLE 13: Hybridization of antisense oligonucleotide probes to transfected COS-7 cells.

Hybridization was performed as described in Methods. The NPY Y5 probe hybridizes only to the cells transfected with the Y5 cDNA. ND=not done.

Cells	Mock	rY1	rY2	rY4	rY5
Oligo					
rY1	-	+	-	ND	ND
rY2	-	-	+	-	-
rY4	-	-	-	+	-
rY5	-	-	-	-	+

In vivo studies with Y5-selective compounds

The results reported above strongly support a role for the Y5 receptor in regulating feeding behavior. Accordingly, the binding and functional properties of several newly synthesized compounds at the cloned human Y1, human Y2, human Y4, and human Y5 receptors was evaluated.

Table 14 discloses several compounds which bind selectively to the human Y5 receptor and act as Y5 receptor antagonists, as measured by their ability to block NPY-induced inhibition of cAMP accumulation in forskolin-stimulated LM(tk-) cells stably transfected with the cloned human Y5 receptor. The structures of the compounds described in Table 13 are shown in Figure 22. Preliminary experiments indicate that compound 28 is a Y5 receptor antagonist.

Table 14: Evaluation of human Y5 receptor antagonists

The ability of the compounds to antagonize the Y-type receptors is reported as the K_b. The K_b is derived from the EC_{50}, or concentration of half-maximal effect, in the presence (EC_{50}) or absence (EC_{50}') of compound, according to the equation: $K_b = [NPY]/((EC_{50}/EC_{50}')-1)$. The results shown are representative of at least three independent experiments. N.D. = Not determined.

Table 14

	Binding Affinity (K_i (nM) vs. ^{125}I-PYY)				
Compound	Human Receptor				K_b (nM)
-	Y1	Y2	Y4	Y5	-
1	1660	1920	4540	38.9	183
2	1806	386	1280	17.8	9.6
5	3860	249	2290	1.27	2.1
6	4360	4610	32,900	47.5	93
7	2170	2870	7050	42.0	105
9	3240	>100,000	3720	108	479
10	1070	>100,000	5830	40.7	2.8
11	1180	>100,000	7130	9.66	1.5
17	5550	1000	8020	14	6.0
19	3550	955	11700	11	23
20	16000	7760	20400	8.3	26
21	13000	1610	18500	9.8	16
22	17200	7570	27500	11	3.0
23	14500	617	21500	26	38
25	3240	851	13100	17	311

	Binding Affinity (K_i (nM) vs. ^{125}I-PYY)				
26	23700	58200	19300	14	50
27	48700	5280	63100	28	49
28	>100,000	>75,000	>100,000	19,000	N.D.

Several of these compounds were further tested using *in vivo* animal models of feeding behavior.

Since NPY is the strongest known stimulant of feeding behavior, experiments were performed with several compounds to evaluate the effect of the compounds described above on NPY-induced feeding behavior in satiated rats.

First, 300 pmole of porcine NPY in vehicle (ACSF) was administered by intracerebroventricular (i.c.v.) injection, along with i.p. administration of compound vehicle (10% DMSO/water), and the food intake of NPY-stimulated animals was compared to food intake in animals treated with the vehicles. The 300 pmole injection of NPY was found to significantly induce food intake ($p < 0.05$; Student-Newman-Keuls).

Using the 300 pmole dose of NPY found to be effective to stimulate feeding, other animals were treated with the compounds by intraperitoneal (i.p.) administration, followed 30-60 minutes later by i.c.v. NPY administration, and measurement of subsequent food intake. As shown in Table 15, NPY-induced food intake was significantly reduced in animals first treated with the compounds ($p < 0.05$; Student-Newman-Keuls). These experiments demonstrate that NPY-induced food intake is significantly reduced by administration to animals of a compound which is a Y5-selective antagonist.

Table 14 continued

Table 15. NPY-induced cumulative food intake in rats treated with either the i.c.v. and i.p. vehicles (control), 300 pmole NPY alone (NPY), or in rats treated first with compound and then NPY (NPY + compound). Food intake was measured 4 hours after stimulation with NPY. Food intake is reported as the mean ± S.E.M. intake for a group of animals.

Table 15

	Food intake (g) mean ± S.E.M.			
Compound	1	5	17	19
Compound Dose (mg/kg i.p.)	10	10	10	30
control (vehicles only)	3.7 ± 0.6	2.4 ± 0.5	2.4 ± 0.7	2.9 ± 0.8
NPY	7.4 ± 0.5	6.8 ± 1.0	5.8 ± 0.5	4.9 ± 0.4
NPY + compound	4.6 ± 0.6	4.1 ± 0.4	3.8 ± 0.4	1.5 ± 0.6

Since food deprivation induces an increase in the hypothalamic NPY levels, it has been postulated that food intake following a period of food deprivation is NPY-mediated. Therefore, the Y5 antagonists of Table 14 were administered by intraperitoneal injection at a dose of 30 mg/kg to conscious rats following a 24h food deprivation. The human Y5 receptor antagonists shown in Table 14 reduced food intake in the food-deprived animals, as shown below in Table 16. The food intake of animals treated with test compound is reported as the percentage of the food intake measured for control animals (treated with vehicle), i.e., 25% means the animals treated with

the compound consumed only 25% as much food as the control animals. Measurements were performed two hours after administration of the test compound.

Table 16 **Two-hour food intake of food-deprived rats.** Food intake is expressed as the percentage of intake compared to control rats. N.D.= Not done.

Compound	Mean (%)	Compound	Mean (%)
1	34	**19**	36
2	42	**20**	35
5	87	**21**	80
6	38	**22**	55
7	47	**23**	58
9	40	**25**	32
10	74	**26**	73
11	15	**27**	84
17	27	**28**	ND

These experiments indicate that the compounds of the present invention inhibit food intake in rats, especially when administered in a range of about 0.01 to about 100 mg/kg rat, by either oral, intraperitoneal or intravenous administration. The animals appeared normal during these experiments, and no ill effects on the animals were observed after the termination of the feeding experiments.

The binding properties of the compounds were also evaluated with respect to other cloned human G-protein

coupled receptors. As shown in Table 17, below, the Y5-selective compounds described hereinabove exhibited lower affinity for receptors other than the Y-type receptors.

Table 17 Cross-reactivity of compounds at other cloned human receptors

Compound	Receptor (pKi)								
	α_{1d}	α_{1b}	α_{1a}	α_{2a}	α_{2b}	α_{2c}	H1	H2	D3
1	6.25	6.23	6.15	6.28	6.01	6.34	5.59	6.32	5.69
2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
5	7.24	7.36	7.63	7.39	7.29	7.63	6.65	6.68	7.24
6	5.68	5.73	6.54	7.14	5.79	6.35	N.D.	N.D.	N.D.
7	6.46	6.08	6.06	7.16	6.09	6.85	N.D.	N.D.	N.D.
9	6.45	6.26	6.57	7.04	5.00	6.81	N.D.	N.D.	N.D.
10	6.12	5.82	6.27	8.94	5.62	6.18	N.D.	N.D.	N.D.
11	7.03	5.6	6.05	7.38	5.60	6.00	N.D.	N.D.	N.D.
17	6.68	7.17	7.08	6.52	6.51	7.07	6.33	5.92	6.61
19	6.90	7.35	7.47	6.74	6.58	7.07	7.04	6.29	6.69
20	7.01	7.22	7.72	7.31	6.96	7.39	6.73	5.85	6.35
21	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
22	6.80	6.98	7.34	7.05	6.43	7.15	6.22	5.72	6.29
23	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
25	6.66	6.67	7.07	6.21	5.95	6.79	6.43	6.43	5.93
26	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
27	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Table 17 continued

Compound	Receptor (pKi)						
	$5HT_{1a}$	$5HT_2$	$5HT_7$	$5HT_{1F}$	$5HT_{1E}$	$5HT_{1D\beta}$	$5HT_{1D\alpha}$
1	4.51	6.34	6.20	5.30	5.30	5.30	5.42
2	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D
5	6.33	6.41	6.00	5.30	5.30	5.55	5.37
6	N.D.	N.D.	6.00	5.30	5.30	5.30	5.30
7	N.D.	N.D.	6.64	5.30	5.30	5.30	5.85
9	N.D.	N.D.	6.48	5.30	5.30	5.30	5.30
10	N.D.	N.D.	5.87	5.30	5.30	5.30	5.30
11	N.D.	N.D.	6.20	5.30	5.30	5.30	5.30
17	5.88	6.74	6.50	5.30	5.30	5.30	5.32
19	5.54	6.55	6.42	5.30	5.30	5.30	6.04
20	6.73	5.93	6.37	5.30	5.30	5.37	5.94
21	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
22	6.56	5.99	6.39	5.30	5.30	5.41	5.98
23	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
25	5.82	5.99	5.35	5.30	5.30	5.39	5.62
26	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
27	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

EXPERIMENTAL DISCUSSION

In order to isolate new NPY receptor subtypes an expression cloning approach was chosen where a functional receptor is actually detected with exquisite sensitivity on the surface of transfected cells, using a highly specific iodinated ligand. Using this strategy, a rat hypothalamic cDNA encoding a novel Y-type receptor (Y5) was identified. The fact that 3.5 x 10^6 independent clones with a 2.7 kb average insert size had to be screened to find two clones reveals either a very strong bias against Y5 cDNA cloning in the cDNA library construction procedure or that the Y5 mRNA is expressed at very low levels in rat hypothalamic tissue. The longest reading frame in the rat Y5 cDNA (CG-18) encodes a 456 amino acid protein with an estimated molecular weight of 50.1 kD. Given there are two N-linked glycosylation sites in the amino terminus, the apparent molecular weight could be slightly higher. The human Y5 homolog was isolated from a human hippocampal cDNA library. The longest reading frame in the human Y5 cDNA (CG-19) encodes a 455 amino acid protein with an estimated molecular weight of 50 kD. The human Y5 receptor is one amino acid shorter than the rat Y5 and shows significant amino acid differences both in the N-terminal and the middle of the third intracellular loop portions of the protein. The seven transmembrane domains and the extracellular loops, however, are virtually identical and the protein motifs found in both species homologs are identical. Both human and rat Y5 receptors carry a large number of potential phosphorylation sites in their second and third intra- cellular loops which could be involved in the regulation of their functional characteristics.

The rat and human Y5 receptors both carry a leucine zipper in the first putative transmembrane domain. In

such a structure, it has been proposed that segments containing periodic arrays of leucine residues exist in an alpha-helical conformation. The leucine side chains extending from one alpha-helix interact with those from a similar alpha helix of a second polypeptide, facilitating dimerization by the formation of a coiled coil (O'Shea et al, 1989). Usually, such patterns are associated with nuclear DNA binding protein like c-myc, c-fos and c-jun, but it is possible that in some proteins the leucine repeat simply facilitates dimerization and has little to do with positioning a DNA-binding region. Further evidence supporting the idea that dimerization of specific seven transmembrane receptors can occur comes from coexpression studies with muscarinic/adrenergic receptors where intermolecular "cross-talk" between chimeric G-protein coupled receptors has been described (Maggio et al., 1993). The tyrosine phosphorylation site found in the middle of this leucine zipper in transmembrane domain one (TM I) could be involved in regulating dimerization of the Y5 receptor. The physiological significance of G-protein coupled receptor dimerization remains to be elucidated, but by analogy with peptide hormone receptors oligomerization, it could be involved in receptor activation and signal transduction (Wells, 1994).

The nucleotide and amino acid sequence analysis of Y5 (rat and human) reveals low identity levels with all 7 TM receptors including the Y1, Y2 and Y4 receptors, even in the transmembrane domains which are usually highly conserved within receptor subfamilies. CG-18 and CG-19 are named "Y5" receptors because of their unique amino acid sequence (87.2% identical with each other, ≤ 42% identical with the TM regions of previously cloned "Y" receptor subtypes) and pharmacological profile. The name is not biased toward

any one member of the pancreatic polypeptide family. Indeed, the ability of the human Y5 receptor to bind all three known members of the pancreatic polypeptide family (human NPY, human PYY and human PP) with similar affinity (Table 8) suggests the concept of a "universal receptor" and provides an argument against using endogenous peptide ligands for pharmacological classification. The "Y" has its roots in the original classification of Y1 and Y2 receptor subtypes (Wahlestedt et al., 1987). The letter reflects the conservation in pancreatic polypeptide family members of the C-terminal tyrosine, described as "Y" in the single letter amino acid code. The number is the next available in the Y-type series, position number three having been reserved for the pharmacologically defined Y3 receptor. It is noted that the cloned human Y1 receptor was introduced by Larhammar and co-workers as a "human neuropeptide Y/peptide YY receptor of the Y1 type" (Larhammar et al., 1992). Similarly, the novel clones described herein can be described as rat, human and canine neuropeptide Y/peptide YY receptors of the Y5 type.

An electronic search of the GenBank database for sequences with similarity to the human Y5 receptor sequences identified a match between the reverse complement of the human Y5 coding sequence and the human Y1 receptor exon IC and its flanking sequences. Exon 1C is located in the 5'-untranslated region of the Y1C alternate splice variant mRNA of the human Y1 receptor (Ball, et al., 1995). This data reveals that the human Y1 and Y5 receptor genes map, in opposite orientation, to the same locus on chromosome 4q (see Figure 25).

In addition, a restriction site polymorphism has been described in the Y1 receptor gene (Herzog, et al.,

1993), 3.1 kb upstream (5') of the Y1 coding sequence and therefore about 21 kb upstream of the Y5 coding region. It was speculated that this polymorphism in the Y1 receptor gene is associated with changes in feeding behavior because subjects homozygous for this allele demonstrate a modified feeding behavior, resulting in small changes in energy intake and macronutrient selection (Cote, et al., 1995). However, the observation that the Y1 and Y5 receptor genes are co-localized on the same locus and that the efficacy of peptides in *in vivo* feeding correlates to their *in vitro* functional activity at the Y5 receptor, suggests that this polymorphism is associated with the Y5 rather than the Y1 gene as was previously speculated. It will be important to characterize the association of this locus with feeding disorders or obesity in human populations.

The rat hypothalamic Y5 receptor displays a very similar pharmacological profile to the pharmacologically described "atypical" Y1 receptor thought to mediate NPY-induced food intake in rat hypothalamus. Both the Y5 receptor and the "feeding receptor" display a preference for NPY and PYY-like analogs, a sensitivity to N-terminal peptide deletion, and a tolerance for Pro^{34}. Each would be considered Y1-like except for the anomalous ability of NPY_{2-36} to bind and activate as well as NPY. Each appears to be sensitive to changes in the mid-region of the peptide ligand. For example, a study by Kalra and colleagues (1991) indicated that replacement of the NPY midregion by an amino-octanoic chain to produce NPY_{1-4}-Aca-$_{25-36}$ dramatically reduced activity in a feeding behavioral assay. Likewise, it is noted that the robust difference in human PP binding (K_i = 5.0 nM) and rat PP binding (K_i = 230) to the rat Y5 receptor can be attributed to a series of 8 amino acid changes between

residues 6-30 in the peptide ligands, with human PP bearing the closer resemblance to human NPY. Further examination of PP ligands indicates that those which are capable of activating the Y5 receptor with high potency, such as bovine and human PP, contain a proline in position 13 or 14. While this proline is conserved in several PP ligands (porcine, sheep, and canine, for example) and also in human and porcine NPY as well as human and porcine PYY, it is not conserved in rat PP. This structural difference may lead to changes in protein folding and ultimately to changes in receptor interaction which underlie the relatively poor potency of rat PP for Y5 receptor activation. The understanding of these structure-activity relationships may be important for the design of Y5 selective ligands with the ability to modulate food intake *in vivo*.

Noted also that FLRFamide, a structural analog of the FMRFamide peptide which is reported to stimulate feeding in rats, was able to bind and activate the rat Y5 receptor albeit at relatively high concentrations (Orosco, et al., 1989). These matching profiles, combined with a selective activation of the rat Y5 by the reported feeding "modulator" [D-Trp^{32}]NPY, support the identity of the rat Y5 as the "feeding receptor" first proposed to explain NPY-induced feeding in rat hypothalamus. That the human Y5 receptor has a pharmacological profile like that of the rat Y5 in both binding and functional assays suggests that the two receptors may have similar functions in vivo.

The distribution of Y5 mRNA in rat brain further extends the argument for a role of Y5 receptors in feeding behavior. The anatomical locus of the feeding response, for example, has been suggested to reside at least in part in the paraventricular hypothalamic nucleus (PVN) and also in the lateral hypothalamus, two

places where Y5 mRNA was detected in abundance. Post-synaptic localization of the Y5 receptor in both of these regions can regulate the response to endogenously released NPY in vivo. The paraventricular nucleus receives projections from NPY-containing neurons in the arcuate nucleus, another region where Y5 mRNA was detected. This indicates a pre-synaptic role for the Y5 receptor in the control of NPY release via the arcuato-paraventricular projection, and consequently in the control of feeding behavior. The localization of the Y5 mRNA in the midline thalamic nuclei is also important. The paraventricular thalamic nucleus/centromedial nucleus complex projects heavily to the paraventricular hypothalamus and to the amygdala. As such, the Y5 receptor is a substrate for the emotional aspect of appetitive behaviors.

Y5 receptors are highly attractive targets for appetite and weight control based on several lines of research (Sahu and Kalra, 1993). NPY is the most potent stimulant of feeding behavior yet described (Clark et al., 1984; Levine and Morley, 1984; Stanley and Leibowitz, 1984). Direct injection of NPY into the hypothalamus of rats can increase food intake ~ 10-fold over a 4-hour period (Stanley et al., 1992). NPY-stimulated rats display a preference for carbohydrates over protein and fat (Stanley et al., 1985). Interestingly, NPY and NPY mRNA are increased in food-deprived rats (Brady et al., 1990; O' Shea and Gundlach, 1991) and also in rats which are genetically obese (Sanacora et al., 1990) or made diabetic by treatment with streptozotocin (White et al., 1990). One potential explanation is that NPY, a potent stimulant of feeding behavior in normal rats, is disregulated in the overweight or diabetic animal so that food intake is increased, accompanied by obesity. The physiological stress of obesity increases the risk

for health problems such as cardiovascular malfunction, osteoarthritis, and hyperinsulinemia, together with a worsened prognosis for adult-onset diabetes. A nonpeptide antagonist targeted to the Y5 receptor could therefore be effective as a way to control not only appetite and body weight but an entire range of obesity- and diabetes-related disorders (Dryden et al., 1994). There is also neurochemical evidence to suggest that NPY-mediated functions are disregulated in eating disorders such as bulimia and anorexia nervosa, so that they too could be responsive to treatment by a Y5-selective drug. It has been proposed, for example, that food intake in NPY-stimulated rats mimics the massive food consumption associated with binge eating in bulimia (Stanley, 1993). Cerebro-spinal fluid (CSF) levels of PYY but not NPY were elevated in bulimic patients who abstained from binging, and then diminished when binging was allowed (Berrettini et al., 1988). Conversely, NPY levels were elevated in underweight anorectic patients and then diminished as body weight was normalized (Kaye et al., 1990).

As described above, the human and rat in vitro expression models were used in combination to screen for compounds intended to modulate NPY-dependent feeding behavior. Using this approach, several compounds were discovered which inhibit feeding behavior in animal models, which should lead to additional drug discoveries.

The characterization of the canine Y5 receptor in porcine ^{125}I-PYY binding assays with human analogs of NPY, PYY and PP provides a logical basis for comparison with the human and rat receptor homologs. The peptides also have relevance in the context of canine physiology. NPY is highly conserved across species (e.g. 100% in human, rat, guinea pig, rabbit and

alligator) such that canine NPY is predicted to resemble human NPY, although the sequence of canine NPY is currently unknown. Canine and human PYY differ in only 2 out of 36 positions, whereas canine PYY is identical to porcine PYY. Finally, human and canine PP deviate in only 2 out of 36 residues. Thus, the canine Y5 receptor appears to be a plausible target not only for NPY synthesized in the canine nervous system, but also for circulating or neurally-derived PYY and PP. Given the general conservation in structure and pharmacology of Y5 receptors, it is hypothesized that the canine Y5 receptor mediates all of the functions proposed for human and rat Y5 receptors, including the stimulation of feeding behavior. The cloned canine Y5 receptor and canine *in vivo* models are therefore believed to comprise a useful system with which to evaluate biological actions of Y5-selective compounds for the treatment of obesity and eating disorders in humans.

The Y5 pharmacological profile further offers a new standard by which to review the molecular basis of all NPY-dependent processes; examples are listed in Table 18. Such an exercise suggests that the Y5 receptor is likely to have a physiological significance beyond feeding behavior. It has been reported, for example, that a Y-type receptor can regulate luteinizing hormone releasing hormone (LHRH) release from the median eminence of steroid-primed rats *in vitro* with an atypical Y1 pharmacological profile. NPY, NPY_{2-36}, and LP-NPY were all effective at 1uM but deletion of as few as four amino acids from the N-terminus of NPY destroyed biological activity. The Y5 may therefore represent a therapeutic target for sexual or reproductive disorders. Preliminary *in situ* hybridization of rat Y5 mRNA in hippocampus and elsewhere further suggest that additional roles will be

uncovered, for example, in the regulation of memory. The localization of Y5 mRNA in amygdala also suggests a potential role for Y5 receptor modulation in affective disorders such as depression and anxiety. It is worth while considering that the Y5 is so similar in pharmacological profile to the other Y-type receptors that it may have been overlooked among a mixed population of Y1, Y2 and Y4 receptors. Certain functions now associated with these subtypes could therefore be reassigned to Y5 as pharmacological tools grow more sophisticated (Table 18). By offering new insight into NPY receptor pharmacology, the Y5 thereby provides a greater clarity and focus in the field of drug design.

TABLE 18: Pathophysiological Conditions Associated With NPY

The following pathological conditions have been linked to either 1) application of exogenous NPY, or 2) changes in levels of endogenous NPY.

1	obesity	Sahu and Kalra, 1993
2	eating disorders (anorexia and bulimia nervosa)	Stanley, 1993
3	sexual/reproductive function	Clark, 1994
4	depression	Heilig and Weiderlov, 1990
5	anxiety	Wahlestedt et al., 1993
6	cocaine addiction	Wahlestedt et al., 1991
7	gastric ulcer	Penner et al., 1993
8	memory loss	Morley and Flood, 1990
9	pain	Hua et al., 1991
10	epileptic seizure	Rizzi et al., 1993
11	hypertension	Zukowska-Grojec et al., 1993
12	subarachnoid hemorrhage	Abel et al., 1988
13	shock	Hauser et al., 1993
14	circadian rhythm	Albers and Ferris, 1984
15	nasal congestion	Lacroix et al., 1988
16	diarrhea	Cox and Cuthbert, 1990
17	neurogenic voiding dysfunction	Zoubek et al., 1993

A successful strategy for the design of a Y5-receptor based drug or for any drug targeted to single G protein-coupled receptor subtype involves the screening of candidate compounds 1) in radioligand binding assays so as to detect affinity for cross-reactive G protein-coupled receptors, and 2) in physiological assays so as to detect undesirable side effects. In the specific process of screening for a Y5-selective drug, the receptor subtypes most likely to cross-react and therefore most important for radioligand binding screens include the other "Y-type" receptors, Y1, Y2, Y3, and Y4. Cross-reactivity between the Y5 and any of the other subtypes could result in potential complications as suggested by the pathophysiological indications listed in Table 18. In designing a Y5 antagonist for obesity and appetite control, for example, it is important not to design a Y1 antagonist resulting in hypertension or increased anxiety, a Y2 antagonist resulting in memory loss, or a Y4 antagonist resulting in increased appetite.

TABLE 19: Y-Type Receptor Indications

Y-type Receptor Indications	Receptor Subtype	Drug Activity	Reference
obesity, appetite disorder	atypical Y1	antagonist	Sahu and Kalra, 1993
adult onset diabetes	atypical Y1	antagonist	Sahu and Kalra, 1993
bulimia nervosa	atypical Y1	antagonist	Stanley, 1993
pheochromocytoma-induced hypertension	Y1	antagonist	Grouzman et al., 1989
subarachnoid hemorrhage	Y1	antagonist	Abel et al., 1988
neurogenic vascular hypertrophy	Y1 Y2	antagonist antagonist	Zukowska-Grojec et al., 1993
epileptic seizure	Y2	antagonist	Rizzi et al., 1993
hypertension: central, peripheral regulation	peripheral Y1 central Y3 central Y2	antagonist agonist antagonist	Grundemar and Hakanson, 1993 Barraco et al., 1991
obesity, appetite disorder	Y4 or PP	agonist	Malaisse-Lagae et al., 1977
anorexia nervosa	atypical Y1	agonist	Berrettini et al., 1988
anxiety	Y1	agonist	Wahlestedt et al., 1993
cocaine addiction	Y1	agonist	Wahlestedt et al., 1991

Table 19 continued

stress-induced gastric ulcer	Y1 Y4 or PP	agonist agonist	Penner et al., 1993
memory loss	Y2	agonist	Morley and Flood, 1990
pain	Y2	agonist	Hua et al., 1991
shock	Y1	agonist	Hauser et al., 1993
sleep disturbances, jet lag	Y2	not clear	Albers and Ferris, 1984
nasal decongestion	Y1 Y2	agonist agonist	Lacroix et al., 1988
diarrhea	Y2	agonist	Cox and Cuthbert, 1990

The cloning of the Y5 receptor from human and rat is especially valuable for receptor characterization based on in situ localization, anti-sense functional knock-out, and gene induction. These studies will generate important information related to Y5 receptor function and its therapeutic significance. The cloned Y5 receptor lends itself to mutagenesis studies in which receptor/ligand interactions can be modeled. The Y5 receptor further allows us to investigate the possibility of other Y-type receptors through homology cloning. These could include new receptor subtypes as well as Y5 species homologs for the establishment of experimental animal models with relevance for human pathology. The Y5 receptor therefore represents an enormous opportunity for the development of novel and selective drug therapies, particularly those targeted to appetite and weight control, but also for memory loss, depression, anxiety, gastric ulcer, epileptic seizure, pain, hypertension, subarachnoid hemorrhage, sleeping disturbances, nasal congestion, neurogenic voiding dysfuncion, and diarrhea.

In particular, the discovery of Y5-selective antagonists which inhibit food intake in rats provides a method of modifying feeding behavior in a wide variety of vertebrate animals.

TABLE 20: Pharmacological profile of the canine Y5 receptor.

IC_{50} values from competitive displacement of porcine ^{125}I-PYY binding to membranes of COS-7 cells transiently transfected with canine Y5 receptor cDNA were converted to K_i values according to the Cheng-Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$. For all peptides, n = 2. For BIBP 3226, n = 3.

Peptide	K_i
NPY, human	2.2
NPY, porcine	6.2
NPY_{2-36}, porcine	2.1
NPY_{3-36}, porcine	16
NPY_{13-36}, porcine	120
$[Leu^{31},Pro^{34}]$NPY, porcine	4.1
C2-NPY, porcine	300
D-$[Trp^{32}]$NPY, human	35
PYY, human	3.2
PYY_{3-36}, human	14
$[Pro^{34}]$PYY, human	1.4
PP, human	6.3
PP, bovine	10
PP, rat	160
BIBP 3226	17000

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: Gerald, Christophe P.G.
Walker, Mary W.
Branchek, Theresa
Weinshank, Richard L.

(ii) TITLE OF INVENTION: METHODS OF MODIFYING FEEDING BEHAVIOR, COMPOUNDS USEFUL IN SUCH METHODS, AND DNA ENCODING A HYPOTHALAMIC ATYPICAL NEUROPEPTIDE Y/PEPTIDE YY RECEPTOR (Y5)

(iii) NUMBER OF SEQUENCES: 24

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Cooper & Dunham LLP
(B) STREET: 1185 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: United States of America
(F) ZIP: 10036

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, John P.
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 1795/46166-C

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 278-0400
(B) TELEFAX: (212) 391-0525

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 61..1432

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GAG GAG CAT TTT AAC AAG ACA TTT GTC ACA GAG AAC AAT ACA GCT GCT     156
Glu Glu His Phe Asn Lys Thr Phe Val Thr Glu Asn Asn Thr Ala Ala
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GCT CGG AAT GCA GCC TTC CCT GCC TGG GAG GAC TAC AGA GGC AGC GTA     204
Ala Arg Asn Ala Ala Phe Pro Ala Trp Glu Asp Tyr Arg Gly Ser Val
        35                  40                  45

GAC GAT TTA CAA TAC TTT CTG ATT GGG CTC TAT ACA TTC GTA AGT CTT     252
Asp Asp Leu Gln Tyr Phe Leu Ile Gly Leu Tyr Thr Phe Val Ser Leu
    50                  55                  60

CTT GGC TTT ATG GGC AAT CTA CTT ATT TTA ATG GCT GTT ATG AAA AAG     300
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65                  70                  75                  80

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Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn Pro
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 457 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Asp Asp Leu Gln Tyr Phe Leu Ile Gly Leu Tyr Thr Phe Val Ser Leu
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Arg Asn Gln Lys Thr Thr Val Asn Phe Leu Ile Gly Asn Leu Ala Phe
85 90 95

Ser Asp Ile Leu Val Val Leu Phe Cys Ser Pro Phe Thr Leu Thr Ser
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Val Leu Leu Asp Gln Trp Met Phe Gly Lys Ala Met Cys His Ile Met
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Cys Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg Ile Ala Phe Thr Ile
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225 230 235 240

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Glu Asn Arg Leu Glu Glu Asn Glu Met Ile Asn Leu Thr Leu Gln Pro
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Ser Lys Val Ile Pro Gly Val Pro Ile Cys Phe Glu Val Lys Pro Glu
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Glu Ser Ser Asp Ala His Glu Met Arg Val Lys Arg Ser Ile Thr Arg
355 360 365

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Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu Ile
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Leu Val Phe Ala Val Ser Trp Met Pro Leu His Val Phe His Val Val
385                 390                 395                 400

Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn Arg His Phe Lys Leu Val
                405                 410                 415

Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn Pro
            420                 425                 430

Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile Lys Ala Asp Leu Arg Ala
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1457 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 61..1432

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Ser Phe Tyr Ser Lys Gln Asp Tyr Asn Met Asp Leu Glu Leu Asp
  1               5                  10                  15

GAG TAT TAT AAC AAG ACA CTT GCC ACA GAG AAT AAT ACT GCT GCC ACT     156
Glu Tyr Tyr Asn Lys Thr Leu Ala Thr Glu Asn Asn Thr Ala Ala Thr
             20                  25                  30

CGG AAT TCT GAT TTC CCA GTC TGG GAT GAC TAT AAA AGC AGT GTA GAT     204
Arg Asn Ser Asp Phe Pro Val Trp Asp Asp Tyr Lys Ser Ser Val Asp
         35                  40                  45

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Asp Leu Gln Tyr Phe Leu Ile Gly Leu Tyr Thr Phe Val Ser Leu Leu
     50                  55                  60

GGC TTT ATG GGG AAT CTA CTT ATT TTA ATG GCT CTC ATG AAA AAG CGT     300
Gly Phe Met Gly Asn Leu Leu Ile Leu Met Ala Leu Met Lys Lys Arg
 65                  70                  75                  80

AAT CAG AAG ACT ACG GTA AAC TTC CTC ATA GGC AAT CTG GCC TTT TCT     348
Asn Gln Lys Thr Thr Val Asn Phe Leu Ile Gly Asn Leu Ala Phe Ser
                 85                  90                  95

GAT ATC TTG GTT GTG CTG TTT TGC TCA CCT TTC ACA CTG ACG TCT GTC     396
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Asp Ile Leu Val Val Leu Phe Cys Ser Pro Phe Thr Leu Thr Ser Val
            100                 105                 110

TTG CTG GAT CAG TGG ATG TTT GGC AAA GTC ATG TGC CAT ATT ATG CCT     444
Leu Leu Asp Gln Trp Met Phe Gly Lys Val Met Cys His Ile Met Pro
        115                 120                 125

TTT CTT CAA TGT GTG TCA GTT TTG GTT TCA ACT TTA ATT TTA ATA TCA     492
Phe Leu Gln Cys Val Ser Val Leu Val Ser Thr Leu Ile Leu Ile Ser
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ATT GCC ATT GTC AGG TAT CAT ATG ATA AAA CAT CCC ATA TCT AAT AAT     540
Ile Ala Ile Val Arg Tyr His Met Ile Lys His Pro Ile Ser Asn Asn
145                 150                 155                 160

TTA ACA GCA AAC CAT GGC TAC TTT CTG ATA GCT ACT GTC TGG ACA CTA     588
Leu Thr Ala Asn His Gly Tyr Phe Leu Ile Ala Thr Val Trp Thr Leu
                165                 170                 175

GGT TTT GCC ATC TGT TCT CCC CTT CCA GTG TTT CAC AGT CTT GTG GAA     636
Gly Phe Ala Ile Cys Ser Pro Leu Pro Val Phe His Ser Leu Val Glu
            180                 185                 190

CTT CAA GAA ACA TTT GGT TCA GCA TTG CTG AGC AGC AGG TAT TTA TGT     684
Leu Gln Glu Thr Phe Gly Ser Ala Leu Leu Ser Ser Arg Tyr Leu Cys
        195                 200                 205

GTT GAG TCA TGG CCA TCT GAT TCA TAC AGA ATT GCC TTT ACT ATC TCT     732
Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg Ile Ala Phe Thr Ile Ser
    210                 215                 220

TTA TTG CTA GTT CAG TAT ATT CTG CCC TTA GTT TGT CTT ACT GTA AGT     780
Leu Leu Leu Val Gln Tyr Ile Leu Pro Leu Val Cys Leu Thr Val Ser
225                 230                 235                 240

CAT ACA AGT GTC TGC AGA AGT ATA AGC TGT GGA TTG TCC AAC AAA GAA     828
His Thr Ser Val Cys Arg Ser Ile Ser Cys Gly Leu Ser Asn Lys Glu
                245                 250                 255

AAC AGA CTT GAA GAA AAT GAG ATG ATC AAC TTA ACT CTT CAT CCA TCC     876
Asn Arg Leu Glu Glu Asn Glu Met Ile Asn Leu Thr Leu His Pro Ser
            260                 265                 270

AAA AAG AGT GGG CCT CAG GTG AAA CTC TCT GGC AGC CAT AAA TGG AGT     924
Lys Lys Ser Gly Pro Gln Val Lys Leu Ser Gly Ser His Lys Trp Ser
        275                 280                 285

TAT TCA TTC ATC AAA AAA CAC AGA AGA AGA TAT AGC AAG AAG ACA GCA     972
Tyr Ser Phe Ile Lys Lys His Arg Arg Arg Tyr Ser Lys Lys Thr Ala
    290                 295                 300

TGT GTG TTA CCT GCT CCA GAA AGA CCT TCT CAA GAG AAC CAC TCC AGA    1020
Cys Val Leu Pro Ala Pro Glu Arg Pro Ser Gln Glu Asn His Ser Arg
305                 310                 315                 320

ATA CTT CCA GAA AAC TTT GGC TCT GTA AGA AGT CAG CTC TCT TCA TCC    1068
Ile Leu Pro Glu Asn Phe Gly Ser Val Arg Ser Gln Leu Ser Ser Ser
                325                 330                 335

AGT AAG TTC ATA CCA GGG GTC CCC ACT TGC TTT GAG ATA AAA CCT GAA    1116
Ser Lys Phe Ile Pro Gly Val Pro Thr Cys Phe Glu Ile Lys Pro Glu
            340                 345                 350

GAA AAT TCA GAT GTT CAT GAA TTG AGA GTA AAA CGT TCT GTT ACA AGA    1164
Glu Asn Ser Asp Val His Glu Leu Arg Val Lys Arg Ser Val Thr Arg
        355                 360                 365
```

```
ATA AAA AAG AGA TCT CGA AGT GTT TTC TAC AGA CTG ACC ATA CTG ATA   1212
Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu Ile
    370                 375                 380

TTA GTA TTT GCT GTT AGT TGG ATG CCA CTA CAC CTT TTC CAT GTG GTA   1260
Leu Val Phe Ala Val Ser Trp Met Pro Leu His Leu Phe His Val Val
385                 390                 395                 400

ACT GAT TTT AAT GAC AAT CTT ATT TCA AAT AGG CAT TTC AAG TTG GTG   1308
Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn Arg His Phe Lys Leu Val
                405                 410                 415

TAT TGC ATT TGT CAT TTG TTG GGC ATG ATG TCC TGT TGT CTT AAT CCA   1356
Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn Pro
            420                 425                 430

ATT CTA TAT GGG TTT CTT AAT AAT GGG ATT AAA GCT GAT TTA GTG TCC   1404
Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile Lys Ala Asp Leu Val Ser
        435                 440                 445

CTT ATA CAC TGT CTT CAT ATG TAA TAA TTCTCACTGT TTACCAAGGA         1452
Leu Ile His Cys Leu His Met  *   *
    450                 455

AAGAAC                                                            1457
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 457 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ser Phe Tyr Ser Lys Gln Asp Tyr Asn Met Asp Leu Glu Leu Asp
 1               5                  10                  15

Glu Tyr Tyr Asn Lys Thr Leu Ala Thr Glu Asn Asn Thr Ala Ala Thr
            20                  25                  30

Arg Asn Ser Asp Phe Pro Val Trp Asp Asp Tyr Lys Ser Ser Val Asp
        35                  40                  45

Asp Leu Gln Tyr Phe Leu Ile Gly Leu Tyr Thr Phe Val Ser Leu Leu
    50                  55                  60

Gly Phe Met Gly Asn Leu Leu Ile Leu Met Ala Leu Met Lys Lys Arg
65                  70                  75                  80

Asn Gln Lys Thr Thr Val Asn Phe Leu Ile Gly Asn Leu Ala Phe Ser
                85                  90                  95

Asp Ile Leu Val Val Leu Phe Cys Ser Pro Phe Thr Leu Thr Ser Val
            100                 105                 110

Leu Leu Asp Gln Trp Met Phe Gly Lys Val Met Cys His Ile Met Pro
        115                 120                 125

Phe Leu Gln Cys Val Ser Val Leu Val Ser Thr Leu Ile Leu Ile Ser
    130                 135                 140

Ile Ala Ile Val Arg Tyr His Met Ile Lys His Pro Ile Ser Asn Asn
145                 150                 155                 160
```

```
Leu Thr Ala Asn His Gly Tyr Phe Leu Ile Ala Thr Val Trp Thr Leu
                165                 170                 175

Gly Phe Ala Ile Cys Ser Pro Leu Pro Val Phe His Ser Leu Val Glu
            180                 185                 190

Leu Gln Glu Thr Phe Gly Ser Ala Leu Leu Ser Ser Arg Tyr Leu Cys
        195                 200                 205

Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg Ile Ala Phe Thr Ile Ser
    210                 215                 220

Leu Leu Leu Val Gln Tyr Ile Leu Pro Leu Val Cys Leu Thr Val Ser
225                 230                 235                 240

His Thr Ser Val Cys Arg Ser Ile Ser Cys Gly Leu Ser Asn Lys Glu
                245                 250                 255

Asn Arg Leu Glu Glu Asn Glu Met Ile Asn Leu Thr Leu His Pro Ser
            260                 265                 270

Lys Lys Ser Gly Pro Gln Val Lys Leu Ser Gly Ser His Lys Trp Ser
        275                 280                 285

Tyr Ser Phe Ile Lys Lys His Arg Arg Arg Tyr Ser Lys Lys Thr Ala
    290                 295                 300

Cys Val Leu Pro Ala Pro Glu Arg Pro Ser Gln Glu Asn His Ser Arg
305                 310                 315                 320

Ile Leu Pro Glu Asn Phe Gly Ser Val Arg Ser Gln Leu Ser Ser Ser
                325                 330                 335

Ser Lys Phe Ile Pro Gly Val Pro Thr Cys Phe Glu Ile Lys Pro Glu
            340                 345                 350

Glu Asn Ser Asp Val His Glu Leu Arg Val Lys Arg Ser Val Thr Arg
        355                 360                 365

Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu Ile
    370                 375                 380

Leu Val Phe Ala Val Ser Trp Met Pro Leu His Leu Phe His Val Val
385                 390                 395                 400

Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn Arg His Phe Lys Leu Val
                405                 410                 415

Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn Pro
            420                 425                 430

Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile Lys Ala Asp Leu Val Ser
        435                 440                 445

Leu Ile His Cys Leu His Met  *   *
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 1054 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..1004

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TC ATG TGT CAC ATT ATG CCT TTT CTT CAA TGT GTG TCA GTT CTG GTT 47
Met Cys His Ile Met Pro Phe Leu Gln Cys Val Ser Val Leu Val
1 5 10 15

TCA ACT TTA ATT CTA ATA TCA ATT GCC ATT GTC AGG TAT CAT ATG ATC 95
Ser Thr Leu Ile Leu Ile Ser Ile Ala Ile Val Arg Tyr His Met Ile
20 25 30

AAG CAT CCT ATA TCT AAC AAT TTA ACA GCA AAC CAT GGC TAC TTC CTG 143
Lys His Pro Ile Ser Asn Asn Leu Thr Ala Asn His Gly Tyr Phe Leu
35 40 45

ATT GCT ACT GTC TGG ACA CTA GGT TTT GCG ATT TGT TCT CCC CTT CCA 191
Ile Ala Thr Val Trp Thr Leu Gly Phe Ala Ile Cys Ser Pro Leu Pro
50 55 60

GTG TTT CAC AGT CTG GTG GAA CTT CAG GAA ACA TTT GAC TCC GCA TTG 239
Val Phe His Ser Leu Val Glu Leu Gln Glu Thr Phe Asp Ser Ala Leu
65 70 75

CTG AGC AGC AGG TAT TTA TGT GTT GAG TCG TGG CCA TCT GAT TCG TAC 287
Leu Ser Ser Arg Tyr Leu Cys Val Glu Ser Trp Pro Ser Asp Ser Tyr
80 85 90 95

AGA ATC GCT TTT ACT ATC TCT TTA TTG CTA GTC CAG TAT ATT CTT CCC 335
Arg Ile Ala Phe Thr Ile Ser Leu Leu Leu Val Gln Tyr Ile Leu Pro
100 105 110

TTG GTG TGT CTA ACT GTG AGC CAT ACC AGT GTC TGC AGG AGT ATA AGC 383
Leu Val Cys Leu Thr Val Ser His Thr Ser Val Cys Arg Ser Ile Ser
115 120 125

TGC GGG TTG TCC AAC AAA GAA AAC AAA CTG GAA GAA AAC GAG ATG ATC 431
Cys Gly Leu Ser Asn Lys Glu Asn Lys Leu Glu Glu Asn Glu Met Ile
130 135 140

AAC TTA ACT CTT CAA CCA TTC AAA AAG AGT GGG CCT CAG GTG AAA CTT 479
Asn Leu Thr Leu Gln Pro Phe Lys Lys Ser Gly Pro Gln Val Lys Leu
145 150 155

TCC AGC AGC CAT AAA TGG AGC TAT TCA TTC ATC AGA AAA CAC AGG AGA 527
Ser Ser Ser His Lys Trp Ser Tyr Ser Phe Ile Arg Lys His Arg Arg
160 165 170 175

AGG TAC AGC AAG AAG ACG GCG TGT GTC TTA CCT GCT CCA GCA AGA CCT 575
Arg Tyr Ser Lys Lys Thr Ala Cys Val Leu Pro Ala Pro Ala Arg Pro
180 185 190

CCT CAA GAG AAC CAC TCA AGA ATG CTT CCA GAA AAC TTT GGT TCT GTA 623
Pro Gln Glu Asn His Ser Arg Met Leu Pro Glu Asn Phe Gly Ser Val
195 200 205

AGA AGT CAG CAT TCT TCA TCC AGT AAG TTC ATA CCG GGG GTC CCC ACC 671
Arg Ser Gln His Ser Ser Ser Ser Lys Phe Ile Pro Gly Val Pro Thr
210 215 220

TGC TTT GAG GTG AAA CCT GAA GAA AAC TCG GAT GTT CAT GAC ATG AGA 719
Cys Phe Glu Val Lys Pro Glu Glu Asn Ser Asp Val His Asp Met Arg
225 230 235

```
GTA AAC CGT TCT ATC ATG AGA ATC AAA AAG AGA TCC CGA AGT GTT TTC     767
Val Asn Arg Ser Ile Met Arg Ile Lys Lys Arg Ser Arg Ser Val Phe
240                 245                 250                 255

TAT AGA CTA ACC ATA CTG ATA CTA GTG TTT GCC GTT AGC TGG ATG CCA     815
Tyr Arg Leu Thr Ile Leu Ile Leu Val Phe Ala Val Ser Trp Met Pro
                260                 265                 270

CTA CAC CTT TTC CAT GTG GTA ACT GAT TTT AAT GAC AAC CTC ATT TCA     863
Leu His Leu Phe His Val Val Thr Asp Phe Asn Asp Asn Leu Ile Ser
            275                 280                 285

AAC AGG CAT TTC AAA TTG GTG TAT TGC ATT TGT CAT TTG TTA GGC ATG     911
Asn Arg His Phe Lys Leu Val Tyr Cys Ile Cys His Leu Leu Gly Met
        290                 295                 300

ATG TCC TGT TGT CTT AAT CCT ATT CTG TAT GGT TTT CTC AAT AAT GGG     959
Met Ser Cys Cys Leu Asn Pro Ile Leu Tyr Gly Phe Leu Asn Asn Gly
    305                 310                 315

ATC AAA GCT GAT TTA ATT TCC CTT ATA CAG TGT CTT CAT ATG TCA        1004
Ile Lys Ala Asp Leu Ile Ser Leu Ile Gln Cys Leu His Met Ser
320                 325                 330

TAATTATTAA TGTTTACCAA GGAGACAACA AATGTTGGGA TCGTCTAAAA             1054
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 334 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Cys His Ile Met Pro Phe Leu Gln Cys Val Ser Val Leu Val Ser
1               5                   10                  15

Thr Leu Ile Leu Ile Ser Ile Ala Ile Val Arg Tyr His Met Ile Lys
            20                  25                  30

His Pro Ile Ser Asn Asn Leu Thr Ala Asn His Gly Tyr Phe Leu Ile
        35                  40                  45

Ala Thr Val Trp Thr Leu Gly Phe Ala Ile Cys Ser Pro Leu Pro Val
    50                  55                  60

Phe His Ser Leu Val Glu Leu Gln Glu Thr Phe Asp Ser Ala Leu Leu
65                  70                  75                  80

Ser Ser Arg Tyr Leu Cys Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg
                85                  90                  95

Ile Ala Phe Thr Ile Ser Leu Leu Leu Val Gln Tyr Ile Leu Pro Leu
            100                 105                 110

Val Cys Leu Thr Val Ser His Thr Ser Val Cys Arg Ser Ile Ser Cys
        115                 120                 125

Gly Leu Ser Asn Lys Glu Asn Lys Leu Glu Glu Asn Glu Met Ile Asn
    130                 135                 140

Leu Thr Leu Gln Pro Phe Lys Lys Ser Gly Pro Gln Val Lys Leu Ser
145                 150                 155                 160
```

Ser Ser His Lys Trp Ser Tyr Ser Phe Ile Arg Lys His Arg Arg Arg
165 170 175

Tyr Ser Lys Lys Thr Ala Cys Val Leu Pro Ala Pro Ala Arg Pro Pro
180 185 190

Gln Glu Asn His Ser Arg Met Leu Pro Glu Asn Phe Gly Ser Val Arg
195 200 205

Ser Gln His Ser Ser Ser Ser Lys Phe Ile Pro Gly Val Pro Thr Cys
210 215 220

Phe Glu Val Lys Pro Glu Glu Asn Ser Asp Val His Asp Met Arg Val
225 230 235 240

Asn Arg Ser Ile Met Arg Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr
245 250 255

Arg Leu Thr Ile Leu Ile Leu Val Phe Ala Val Ser Trp Met Pro Leu
260 265 270

His Leu Phe His Val Val Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn
275 280 285

Arg His Phe Lys Leu Val Tyr Cys Ile Cys His Leu Leu Gly Met Met
290 295 300

Ser Cys Cys Leu Asn Pro Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile
305 310 315 320

Lys Ala Asp Leu Ile Ser Leu Ile Gln Cys Leu His Met Ser
325 330

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGATCAGTG GATGTTTGGC AAAG 24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTCTGTAGAA AACACTTCGA GATCTCTT 28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTTCCAGTGT TTCACAGTCT GGTGG 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CTGAGCAGCA GGTATTTATG TGTTG 25

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTGGATGAAG AATGCTGACT TCTTAGAG 28

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TTCTTGAGTG GTTCTCTTGA GGAGG 25

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1479 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 62..1432

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GTAGTCTCCC TCTCAGAATT GATTTATCGT AGTCATGTAA TTTTTTAAAA GTTGGTAACT   60

A ATG TCT TTT TAT TCC AAG CAG AAC TCT AAG ATG GAT TTA GAA CTC      106
  Met Ser Phe Tyr Ser Lys Gln Asn Ser Lys Met Asp Leu Glu Leu
    1               5                  10                  15

CAG GAT TTT TAT AAC AAG ACA CTT GCC ACA GAG AAC AAT ACG GCT GCC    154
Gln Asp Phe Tyr Asn Lys Thr Leu Ala Thr Glu Asn Asn Thr Ala Ala
                 20                  25                  30

ACT CGG AAT TCT GAT TTC CCA GTC TGG GAT GAC TAT AAA AGC AGT GTA    202
Thr Arg Asn Ser Asp Phe Pro Val Trp Asp Asp Tyr Lys Ser Ser Val
             35                  40                  45

GAT GAT TTA CAG TAT TTT CTG ATT GGA CTT TAT ACA TTT GTA AGT CTT    250
Asp Asp Leu Gln Tyr Phe Leu Ile Gly Leu Tyr Thr Phe Val Ser Leu
         50                  55                  60

CTC GGT TTT ATG GGG AAT CTA CTT ATT TTA ATG GCT CTC ATG AGA AAG    298
Leu Gly Phe Met Gly Asn Leu Leu Ile Leu Met Ala Leu Met Arg Lys
     65                  70                  75

CGT AAT CAG AAG ACG ATG GTA AAC TTC CTC ATA GGA AAT TTG GCC TTC    346
Arg Asn Gln Lys Thr Met Val Asn Phe Leu Ile Gly Asn Leu Ala Phe
 80                  85                  90                  95

TCT GAT ATT TTG GTT GTG CTG TTT TGC TCA CCT TTT ACA CTG ACC TCT    394
Ser Asp Ile Leu Val Val Leu Phe Cys Ser Pro Phe Thr Leu Thr Ser
                100                 105                 110

GTC CTG CTG GAT CAG TGG ATG TTT GGC AAA GTC ATG TGT CAC ATT ATG    442
Val Leu Leu Asp Gln Trp Met Phe Gly Lys Val Met Cys His Ile Met
            115                 120                 125

CCT TTT CTT CAA TGT GTG TCA GTT CTG GTT TCA ACT TTA ATT CTA ATA    490
Pro Phe Leu Gln Cys Val Ser Val Leu Val Ser Thr Leu Ile Leu Ile
        130                 135                 140

TCA ATT GCC ATT GTC AGG TAT CAT ATG ATC AAG CAT CCT ATA TCT AAT    538
Ser Ile Ala Ile Val Arg Tyr His Met Ile Lys His Pro Ile Ser Asn
    145                 150                 155

AAT TTA ACA GCA AAC CAT GGC TAC TTC CTG ATT GCT ACT GTC TGG ACA    586
Asn Leu Thr Ala Asn His Gly Tyr Phe Leu Ile Ala Thr Val Trp Thr
160                 165                 170                 175

CTA GGT TTT GCG ATT TGT TCT CCC CTT CCA GTG TTT CAC AGT CTG GTG    634
Leu Gly Phe Ala Ile Cys Ser Pro Leu Pro Val Phe His Ser Leu Val
                180                 185                 190

GAA CTT CAG GAA ACA TTT GAC TCC GCA TTG CTG AGC AGC AGG TAT TTA    682
Glu Leu Gln Glu Thr Phe Asp Ser Ala Leu Leu Ser Ser Arg Tyr Leu
            195                 200                 205

TGT GTT GAG TCG TGG CCA TCT GAT TCG TAC AGA ATC GCT TTT ACT ATC    730
Cys Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg Ile Ala Phe Thr Ile
        210                 215                 220

TCT TTA TTG CTA GTC CAG TAT ATT CTT CCC TTG GTG TGT CTA ACT GTG    778
Ser Leu Leu Leu Val Gln Tyr Ile Leu Pro Leu Val Cys Leu Thr Val
    225                 230                 235

AGC CAT ACC AGT GTC TGC AGG AGT ATA AGC TGC GGG TTG TCC AAC AAA    826
Ser His Thr Ser Val Cys Arg Ser Ile Ser Cys Gly Leu Ser Asn Lys
240                 245                 250                 255
```

```
GAA AAC AAA CTG GAA GAA AAC GAG ATG ATC AAC TTA ACT CTT CAA CCA      874
Glu Asn Lys Leu Glu Glu Asn Glu Met Ile Asn Leu Thr Leu Gln Pro
                260                 265                 270

TTC AAA AAG AGT GGG CCT CAG GTG AAA CTT TCC AGC AGC CAT AAA TGG      922
Phe Lys Lys Ser Gly Pro Gln Val Lys Leu Ser Ser Ser His Lys Trp
            275                 280                 285

AGC TAT TCA TTC ATC AGA AAA CAC AGG AGA AGG TAC AGC AAG AAG ACG      970
Ser Tyr Ser Phe Ile Arg Lys His Arg Arg Arg Tyr Ser Lys Lys Thr
        290                 295                 300

GCG TGT GTC TTA CCT GCT CCA GCA AGA CCT CCT CAA GAG AAC CAC TCA     1018
Ala Cys Val Leu Pro Ala Pro Ala Arg Pro Pro Gln Glu Asn His Ser
    305                 310                 315

AGA ATG CTT CCA GAA AAC TTT GGT TCT GTA AGA AGT CAG CAT TCT TCA     1066
Arg Met Leu Pro Glu Asn Phe Gly Ser Val Arg Ser Gln His Ser Ser
320                 325                 330                 335

TCC AGT AAG TTC ATA CCG GGG GTC CCC ACC TGC TTT GAG GTG AAA CCT     1114
Ser Ser Lys Phe Ile Pro Gly Val Pro Thr Cys Phe Glu Val Lys Pro
                340                 345                 350

GAA GAA AAC TCG GAT GTT CAT GAC ATG AGA GTA AAC CGT TCT ATC ATG     1162
Glu Glu Asn Ser Asp Val His Asp Met Arg Val Asn Arg Ser Ile Met
            355                 360                 365

AGA ATC AAA AAG AGA TCC CGA AGT GTT TTC TAT AGA CTA ACC ATA CTG     1210
Arg Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu
        370                 375                 380

ATA CTA GTG TTT GCC GTT AGC TGG ATG CCA CTA CAC CTT TTC CAT GTG     1258
Ile Leu Val Phe Ala Val Ser Trp Met Pro Leu His Leu Phe His Val
    385                 390                 395

GTA ACT GAT TTT AAT GAC AAC CTC ATT TCA AAC AGG CAT TTC AAA TTG     1306
Val Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn Arg His Phe Lys Leu
400                 405                 410                 415

GTG TAT TGC ATT TGT CAT TTG TTA GGC ATG ATG TCC TGT TGT CTT AAT     1354
Val Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn
                420                 425                 430

CCT ATT CTG TAT GGT TTT CTC AAT AAT GGG ATC AAA GCT GAT TTA ATT     1402
Pro Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile Lys Ala Asp Leu Ile
            435                 440                 445

TCC CTT ATA CAG TGT CTT CAT ATG TCA TAA TTCTTCATGT TTACCAAGGA       1452
Ser Leu Ile Gln Cys Leu His Met Ser  *
        450                 455

GACAACAAAT GTTGGGATCG TCTAAAA                                       1479
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 457 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Ser Phe Tyr Ser Lys Gln Asn Ser Lys Met Asp Leu Glu Leu Gln
```

1 5 10 15

Asp Phe Tyr Asn Lys Thr Leu Ala Thr Glu Asn Asn Thr Ala Ala Thr
20 25 30

Arg Asn Ser Asp Phe Pro Val Trp Asp Asp Tyr Lys Ser Ser Val Asp
35 40 45

Asp Leu Gln Tyr Phe Leu Ile Gly Leu Tyr Thr Phe Val Ser Leu Leu
50 55 60

Gly Phe Met Gly Asn Leu Leu Ile Leu Met Ala Leu Met Arg Lys Arg
65 70 75 80

Asn Gln Lys Thr Met Val Asn Phe Leu Ile Gly Asn Leu Ala Phe Ser
85 90 95

Asp Ile Leu Val Val Leu Phe Cys Ser Pro Phe Thr Leu Thr Ser Val
100 105 110

Leu Leu Asp Gln Trp Met Phe Gly Lys Val Met Cys His Ile Met Pro
115 120 125

Phe Leu Gln Cys Val Ser Val Leu Val Ser Thr Leu Ile Leu Ile Ser
130 135 140

Ile Ala Ile Val Arg Tyr His Met Ile Lys His Pro Ile Ser Asn Asn
145 150 155 160

Leu Thr Ala Asn His Gly Tyr Phe Leu Ile Ala Thr Val Trp Thr Leu
165 170 175

Gly Phe Ala Ile Cys Ser Pro Leu Pro Val Phe His Ser Leu Val Glu
180 185 190

Leu Gln Glu Thr Phe Asp Ser Ala Leu Leu Ser Ser Arg Tyr Leu Cys
195 200 205

Val Glu Ser Trp Pro Ser Asp Ser Tyr Arg Ile Ala Phe Thr Ile Ser
210 215 220

Leu Leu Leu Val Gln Tyr Ile Leu Pro Leu Val Cys Leu Thr Val Ser
225 230 235 240

His Thr Ser Val Cys Arg Ser Ile Ser Cys Gly Leu Ser Asn Lys Glu
245 250 255

Asn Lys Leu Glu Glu Asn Glu Met Ile Asn Leu Thr Leu Gln Pro Phe
260 265 270

Lys Lys Ser Gly Pro Gln Val Lys Leu Ser Ser Ser His Lys Trp Ser
275 280 285

Tyr Ser Phe Ile Arg Lys His Arg Arg Arg Tyr Ser Lys Lys Thr Ala
290 295 300

Cys Val Leu Pro Ala Pro Ala Arg Pro Pro Gln Glu Asn His Ser Arg
305 310 315 320

Met Leu Pro Glu Asn Phe Gly Ser Val Arg Ser Gln His Ser Ser Ser
325 330 335

Ser Lys Phe Ile Pro Gly Val Pro Thr Cys Phe Glu Val Lys Pro Glu
340 345 350

Glu Asn Ser Asp Val His Asp Met Arg Val Asn Arg Ser Ile Met Arg
355 360 365

```
Ile Lys Lys Arg Ser Arg Ser Val Phe Tyr Arg Leu Thr Ile Leu Ile
    370                 375                 380

Leu Val Phe Ala Val Ser Trp Met Pro Leu His Leu Phe His Val Val
385                 390                 395                 400

Thr Asp Phe Asn Asp Asn Leu Ile Ser Asn Arg His Phe Lys Leu Val
                405                 410                 415

Tyr Cys Ile Cys His Leu Leu Gly Met Met Ser Cys Cys Leu Asn Pro
            420                 425                 430

Ile Leu Tyr Gly Phe Leu Asn Asn Gly Ile Lys Ala Asp Leu Ile Ser
        435                 440                 445

Leu Ile Gln Cys Leu His Met Ser  *
    450                 455
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCCTTTTCTT CAATGTGTGT CAG 23

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCAGACAGTA GCAATCAGGA AGTAGC 26

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AAGCTTCTAG AGATCCCTCG ACCTC 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AGGCGCAGAA CTGGTAGGTA TGGAA 25

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GAACTCTAAG ATGGATTTAG AACTCCAGAT TTT 33

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATGCTTCCGG CTCGTATGTT GTGTGG 26

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCCTCTTCGC TATTACGCCA GCTGGC 26

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TAGTCATCCC AGACTGGG 18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTAGTCTCCC TCTCAGAATT GATTTATCG 29

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGTAAACATG AAGAATTATG ACATATGAAG AC 32

What is claimed is:

1. An isolated nucleic acid encoding a canine Y5 receptor.

2. The nucleic acid of claim 1, wherein the nucleic acid is DNA, RNA, cDNA, mRNA, or genomic DNA.

3. The nucleic acid molecule of claim 1, wherein the canine Y5 receptor has substantially the same amino acid sequence as that shown in Figure 24.

4. The nucleic acid of claim 1, wherein the canine Y5 receptor has the amino acid sequence shown in Figure 24.

5. A purified canine Y5 receptor protein.

6. A vector comprising the nucleic acid of claim 1.

7. A vector of claim 6 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a canine Y5 receptor as to permit expression thereof.

8. A vector of claim 6 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a canine Y5 receptor as to permit expression thereof.

9. A vector of claim 6 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the

insect cell operatively linked to the nucleic acid encoding the canine Y5 receptor as to permit expression thereof.

10. A vector of claim 6 which is a baculovirus.

11. A vector of claim 6 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell operatively linked to the DNA encoding a canine Y5 receptor as to permit expression thereof.

12. A vector of claim 11, wherein the vector is a plasmid.

13. The vector of claim 6 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell operatively linked to the DNA encoding the canine Y5 receptor as to permit expression thereof.

14. The vector of claim 13, wherein the vector is a plasmid.

15. The plasmid of claim 14 designated cY5-BO11 (ATCC Accession No. 97587).

16. A mammalian cell comprising the vector of claim 11, 12, 13, 14, or 15.

17. A mammalian cell of claim 16, wherein the cell is non-neuronal in origin.

18. A mammalian cell of claim 16, wherein the mammalian cell is a COS-7 cell, a CHO cell, the glial cell C6,

a 293 human embryonic kidney cell, a NIH-3T3 cell, or a LM(tk-) cell.

19. An insect cell comprising the vector of claim 9.

20. An insect cell of claim 19, wherein the insect cell is an Sf9 cell.

21. An insect cell of claim 19, wherein the insect cell is an Sf21 cell.

22. A membrane preparation isolated from the cell of claim 16, 19, 20, or 21.

23. A nucleic acid probe comprising a nucleic acid of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the sequence of a nucleic acid encoding a canine Y5 receptor of claim 1.

24. A nucleic acid probe of claim 23, wherein the nucleic acid is DNA.

25. A nucleic acid probe of claim 23, wherein the nucleic acid is RNA.

26. An antisense oligonucleotide having a sequence capable of specifically hybridizing to mRNA encoding a canine Y5 receptor of claim 1 so as to prevent translation of the mRNA.

27. An antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 2.

28. An antisense oligonucleotide of claim 26 or 27, wherein the oligonucleotide comprises chemically

modified nucleotides or nucleotide analogues.

29. An antibody capable of binding to a canine Y5 receptor of claim 5.

30. An antibody capable of competitively inhibiting the binding of the antibody of claim 29 to a canine Y5 receptor.

31. An antibody of claim 29, wherein the antibody is a monoclonal antibody.

32. A monoclonal antibody of claim 31 directed to an epitope of a canine Y5 receptor present on the surface of a canine Y5 receptor expressing cell.

33. A pharmaceutical composition comprising an amount of the oligonucleotide of claim 26 capable of passing through a cell membrane effective to reduce expression of a canine Y5 receptor and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

34. A pharmaceutical composition of claim 33, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

35. A pharmaceutical composition of claim 34, wherein the substance which inactivates mRNA is a ribozyme.

36. A pharmaceutical composition of claim 33, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by the cells after binding to the structure.

37. A pharmaceutical composition of claim 36, wherein

the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

38. A pharmaceutical composition which comprises an amount of the antibody of claim 29 effective to block binding of a ligand to the canine Y5 receptor and a pharmaceutically acceptable carrier.

39. A transgenic nonhuman mammal expressing nucleic acid encoding a canine Y5 receptor of claim 1.

40. A transgenic nonhuman mammal comprising a homologous recombination knockout of the native canine Y5 receptor.

41. A transgenic nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a canine Y5 receptor of claim 2 so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a Y5 receptor and which hybridizes to mRNA encoding a Y5 receptor thereby reducing its translation.

42. The transgenic nonhuman mammal of claim 39 or 40, wherein the DNA encoding a canine Y5 receptor additionally comprises an inducible promoter.

43. The transgenic nonhuman mammal of claim 39 or 40, wherein the DNA encoding a canine Y5 receptor additionally comprises tissue specific regulatory elements.

44. A transgenic nonhuman mammal of claim 39, 40, 41, 42, or 43, wherein the transgenic nonhuman mammal is a mouse.

45. A process for identifying a chemical compound which specifically binds to a Y5 receptor, which comprises contacting nonneuronal cells expressing on their cell surface the Y5 receptor, with the chemical compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the Y5 receptor.

46. A process for identifying a chemical compound which specifically binds to a Y5 receptor, which comprises contacting a membrane fraction from a cell extract of nonneuronal cells expressing on their cell surface the Y5 receptor, with the chemical compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the Y5 receptor.

47. A process involving competitive binding for identifying a chemical compound which specifically binds to a Y5 receptor, which comprises separately contacting nonneuronal cells expressing on their cell surface the Y5 receptor, with both the chemical compound and a second chemical compound known to bind to the Y5 receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the Y5 receptor, a decrease in binding of the second chemical compound to the Y5 receptor in the presence of the chemical compound indicating that the chemical compound binds to the Y5 receptor.

48. A process involving competitive binding for identifying a chemical compound which specifically binds to a Y5 receptor, which comprises separately contacting a membrane fraction from a cell extract of nonneuronal cells expressing on their cell

surface the Y5 receptor, with both the chemical compound and a second chemical compound known to bind to the Y5 receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the Y5 receptor, a decrease in binding of the second chemical compound to the Y5 receptor in the presence of the chemical compound indicating that the chemical compound binds to the Y5 receptor.

49. A process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a Y5 receptor, with the chemical compound under conditions suitable for activation of the Y5 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in second messenger response in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor.

50. A process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting a membrane fraction from a cell extract of nonneuronal cells producing a second messenger response and expressing on their cell surface a Y5 receptor, with the chemical compound under conditions suitable for activation of the Y5 receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in second messenger response in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor.

51. A process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting nonneuronal cells producing a second messenger response and expressing on their cell surface a Y5 receptor, with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the Y5 receptor.

52. A process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting a membrane fraction from a cell extract of nonneuronal cells producing a second messenger response and expressing on their cell surface a Y5 receptor, with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in second messenger response in the presence of both the chemical compound and the second chemical compound than in

the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the Y5 receptor.

53. The process of claim 49 or 50, wherein the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a decrease in adenylate cyclase activity.

54. The process of claim 51 or 52, wherein the second messenger response comprises adenylate cyclase activity and the change in second messenger response is a smaller decrease in the level of adenylate cyclase activity in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

55. The process of either of claims 49 or 50, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger response is an increase in intracellular calcium levels.

56. The process of either of claims 51 or 52, wherein the second messenger response comprises intracellular calcium levels and the change in second messenger response is a smaller increase in the level of intracellular calcium in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

57. The process of claim 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, or 56, wherein the Y5 receptor is a human, a canine, or a rat Y5 receptor.

58. The process of claim 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, or 57, wherein the cell is an insect cell.

59. The process of claim 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, or 58, wherein the cell is a mammalian cell.

60. The process of claim 59, wherein the mammalian cell is a COS-7 cell, a CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

61. The process of claim 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60, wherein the chemical compound is not previously known.

62. A chemical compound identified by the method of claim 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, or 61.

63. A pharmaceutical composition which comprises an amount of chemical compound determined by the process of claim 49 or 50 effective to increase activity of a Y5 receptor and a pharmaceutically acceptable carrier.

64. A pharmaceutical composition of claim 63, wherein the chemical compound is not previously known.

65. A pharmaceutical composition which comprises an amount of a chemical compound determined by the process of either of claims 51 or 52 effective to reduce activity of a Y5 receptor and a pharmaceutically acceptable carrier.

66. A pharmaceutical composition of claim 65, wherein the chemical compound is not previously known.

67. A pharmaceutical composition comprising a chemical compound identified by the process of claim 45, 46, 47, or 48 and a pharmaceutically acceptable carrier.

68. A method of detecting expression of a canine Y5 receptor by detecting the presence of mRNA coding for the Y5 receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with the nucleic acid probe of claim 23 under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the canine Y5 receptor by the cell.

69. A method of treating an abnormality in a subject, wherein the abnormality is alleviated by the inhibition of a canine Y5 receptor which comprises administering to a subject the pharmaceutical composition of claim 33, 34, 35, 36, 37, 38, or 40 in an amount effective to decrease the activity of the canine Y5 receptor in the subject and thereby treat the abnormality.

70. The method of claim 69, wherein the abnormality is obesity.

71. A method of treating an abnormality in a subject wherein the abnormality is alleviated by the activation of a canine Y5 receptor which comprises administering to a subject the pharmaceutical composition of claim 63 or 64, in an amount effective to increase the activation of the canine Y5 receptor in the subject and thereby treat the abnormality.

72. The method of claim 71, wherein the abnormal condition is anorexia.

73. A method of detecting the presence of a canine Y5 receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 29 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a canine Y5 receptor on the surface of the cell.

74. A method of determining the physiological effects of varying levels of activity of cnine Y5 receptors which comprises producing a transgenic nonhuman mammal of claim 39 whose levels of canine Y5 receptor activity are varied by use of an inducible promoter which regulates canine Y5 receptor expression.

75. A method of determining the physiological effects of varying levels of activity of canine Y5 receptors which comprises producing a panel of transgenic nonhuman mammals of claim 39 each expressing a different amount of canine Y5 receptor.

76. A method for identifying an antagonist capable of alleviating an abnormality wherein the abnormality is alleviated by decreasing the activity of a canine Y5 receptor comprising administering the antagonist to the transgenic nonhuman mammal of claim 39, 40, 41, 42, 43, or 44, and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overactivity of a canine Y5 receptor, the alleviation of the abnormality indicating the identification of an antagonist.

77. An antagonist identified by the method of claim 76.

78. A pharmaceutical composition comprising an antagonist identified by the method of claim 76 and a pharmaceutically acceptable carrier.

79. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a canine Y5 receptor which comprises administering to a subject the pharmaceutical composition of claim 78 in an amount effective to decrease the activity of the canine Y5 receptor and thereby treat the abnormality.

80. A method for identifying an agonist capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a canine Y5 receptor comprising administering the agonist to the transgenic nonhuman mammal of claim 39, 40, 41, 42, 43, or 44, and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal, the alleviation of the abnormality indicating the identification of an agonist.

81. An agonist identified by the method of claim 80.

82. A pharmaceutical composition comprising an agonist identified by the method of claim 80 and a pharmaceutically acceptable carrier.

83. A method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a canine Y5 receptor which comprises administering to a subject the pharmaceutical composition of claim 82 in an amount effective to increase the activity of the canine Y5 receptor and thereby treat the abnormality.

84. A method for diagnosing a predisposition to a disorder associated with the activity of a specific allelic form of a canine Y5 receptor which comprises:

a. obtaining DNA from a subject to be tested;

b. digesting the DNA with restriction enzymes;

c. separating the resulting DNA fragments;

d. contacting the fragments with a detectably labeled nucleic acid probe capable of specifically hybridizing with a sequence uniquely present within the sequence of a nucleic acid molecule encoding the allelic form of the canine Y5 receptor; and

e. detecting the presence of labeled probe hybridized to the DNA fragments from the subject being tested, the presence of such hybridized probe indicating that the subject is predisposed to the disorder.

85. A method of preparing the purified canine Y5 receptor of claim 5 which comprises:

a. inducing cells to express the canine Y5 receptor;

b. recovering the receptor from the induced cells; and

c. purifying the receptor so recovered.

86. A method of preparing the purified canine Y5 receptor of claim 5 which comprises:

a. inserting nucleic acid encoding the canine Y5 receptor in a suitable vector;

b. introducing the resulting vector in a suitable host cell;

c. placing the resulting cell in suitable condition permitting production of the isolated canine Y5 receptor;

d. recovering the receptor produced by the resulting cell; and

e. purifying the receptor so recovered.

87. A method for detecting in a subject the presence of a restriction fragment length polymorphism associated with a genomic locus which encompasses both a Y1 and a Y5 receptor gene which comprises:

a. obtaining a sample of DNA from the subject;

b. digesting the DNA with a restriction enzyme;

c. separating the resulting DNA fragments;

d. contacting the DNA fragments with a detectably labeled nucleic acid probe which specifically hybridizes with a sequence uniquely present within the sequence associated with the polymorphism; and

e. detecting whether the probe hybridizes to the DNA fragments, the presence of the labeled probe hybridized to the DNA fragment indicating the presence of the restriction fragment length polymorphism.

88. The method of claim 87, wherein the restriction enzyme is PstI.

89. The method of claim 87, wherein the subject is a human.

90. The method of claim 87 or 88, wherein the polymorphism is associated with susceptibility to modification of feeding behavior using a Y5-selective compound.

91. The method of claim 90, wherein the feeding behavior is anorexia.

92. The method of claim 90, wherein the feeding behavior is bulimia.

93. The method of claim 90, wherein the feeding behavior is associated with obesity.

94. The method of claim 90, 91, 92 or 93, wherein the subject is a human.

95. The method of claim 87, 88, 90, 91, 92 or 93, wherein the subject is an animal.

96. The method of claim 95, wherein the subject is a mammal.

97. The method of claim 96, wherein the subject is a bovine, equine, canine or feline.

98. A method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in anamount effective to inhibit the activity of the subject's Y5 receptor, wherein the binding of the

compound to a human Y5 receptor is characterized by a K_i less than 50 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration and the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor.

99. The method of claim 98, wherein the binding of the compound to each of the human Y1, human Y2, and human Y4 receptors is characterized by a K_i greater than 500 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration.

100. The method of claim 99, wherein the binding of the compound to each of the human Y1, human Y2, and human Y4 receptors is characterized by a K_i greater than 1000 nanomolar.

101. A method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the binding of the compound to the human Y5 receptor is characterized by a K_i less than 5 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration.

102. The method of claim 101, wherein binding of the compound to each of the human Y1, human Y2, and human Y4 receptors is characterized by a K_i greater than 5 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration.

103. The method of claim 101, wherein the compound binds to the human Y5 receptor with an affinity greater

than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor.

104. The method of claim 103, wherein the binding of the compound to each of the human Y1, human Y2 and human Y4 receptors is characterized by a K_i greater than 50 nanomolar when measured in the presence of ^{125}I-PYY at a predetermined concentration.

105. The method of claim 104, wherein the binding of the compound to each of the human Y1, human Y2 and human Y4 receptors is characterized by a K_i greater than 100 nanomolar.

106. A method of treating a subject's feeding disorder which comprises administering to the subject a non-peptidyl compound which is a Y5 receptor antagonist in an amount effective to inhibit the activity of the subject's Y5 receptor, wherein the compound binds to the human Y5 receptor with an affinity greater than ten-fold higher than the affinity with which the compound binds to any other human Y-type receptor.

107. The method of claim 106, wherein the compound binds to the human Y5 receptor with an affinity greater than 26-fold higher than the affinity with which the compound binds to the human Y1 receptor.

108. The method of claim 106, wherein the compound binds to the human Y5 receptor with an affinity greater than 22-fold higher than the affinity with which the compound binds to the human Y2 receptor.

109. The method of claim 106, wherein the compound binds to the human Y5 receptor with an affinity greater

than 34-fold higher than the affinity with which the compound binds to the human Y4 receptor.

110. The method of claim 109, wherein the compound binds to the human Y5 receptor with an affinity greater than 22-fold higher than the affinity with which the compound binds to the human Y2 receptor.

111. The method of claim 110, wherein the compound binds to the human Y5 receptor with an affinity greater than 26-fold higher than the affinity with which the compound binds to the human Y1 receptor.

112. The method of claim 107, wherein the compound binds to the human Y5 receptor with an affinity greater than 100-fold higher than the affinity with which the compound binds to the human Y1 receptor.

113. The method of claim 108, wherein the compound binds to the human Y5 receptor with an affinity greater than 165-fold higher than the affinity with which the compound binds to the human Y2 receptor.

114. The method of claim 109, wherein the compound binds to the human Y5 receptor with an affinity greater than 143-fold higher than the affinity with which the compound binds to the human Y4 receptor.

115. The method of claim 114, wherein the compound binds to the human Y5 receptor with an affinity greater than 165-fold higher than the affinity with which the compound binds to the human Y2 receptor.

116. The method of claim 115, wherein the compound binds to the human Y5 receptor with an affinity greater than 100-fold higher than the affinity with which the compound binds to the human Y1 receptor.

117. The method of claim 116, wherein the compound binds to the human Y5 receptor with an affinity greater than 500-fold higher than the affinity with which the compound binds to each of the human Y1, human Y2, and human Y4 receptors.

118. The method of claim 117, wherein the compound binds to the human Y5 receptor with an affinity greater than 1400-fold higher than the affinity with which the compound binds to each of the human Y1, human Y2, and human Y4 receptors.

119. The method of claim 104 or 106, wherein the feeding disorder is obesity or bulimia.

120. The method of claim 104 or 106, wherein the subject is a vertebrate, a mammal, a human or a canine.

121. A method of decreasing feeding behavior of a subject which comprises, administering to the subject a compound which is a Y5 receptor antagonist and a compound which is a monoamine neurotransmitter uptake inhibitor, wherein the amount of the Y5 antagonist and the monoamine neurotransmitter uptake inhibitor are effective to decrease the feeding behavior of the subject.

122. The method of claim 121, wherein the Y5 antagonist and the monoamine neurotransmitter uptake inhibitor are administered in combination.

123. The method of claim 121, wherein the Y5 antagonist and the monoamine neurotransmitter uptake inhibitor are administered once.

124. The method of claim 121, wherein the Y5 antagonist and the monoamine neurotransmitter uptake inhibitor

are administered separately.

125. The method of claim 124, wherein the Y5 antagonist and the monoamine neurotransmiter uptake inhibitor are administered once.

126. The method of claim 124, wherein the Y5 receptor antagonist is administered for about 2 weeks to about 6 months.

127. The method of claim 124, wherein the monoamine neurotransmitter uptake inhibitor is administered for about 1 month to about 6 months.

128. The method of claim 124, wherein the Y5 antagonist and the monoamine neurotransmitter uptake inhibitor are administered alternately.

129. The method of claim 128, wherin the Y5 antagonist and the monoamine neurotransmitter uptake inhibitor are administered repeatedly.

130. The method of claim 128 or 129, wherein the Y5 receptor antagonist is administered for about 2 weeks to about 6 months.

131. The method of claim 128 or 129, wherein the monoamine neurotransmitter uptake inhibitor is administered for about 1 month to about 6 months.

132. The method of claim 201, wherein the monoamine neurotransmitter uptake inhibitor is administered for about 1 month to about 3 months.

133. The method of claim 121, 122, 123 or 124, wherein the monoamine neurotransmitter uptake inhibitor is a fenfluramine.

134. The method of claim 133, wherein the fenfluramine is dexfenfluramine.

135. The method of claim 121, 122, 123, or 124, wherein the monoamine neurotransmitter uptake inhibitor is sibutramine.

136. The method of claim 121, 122, 123, or 124, wherein the compound is administered in a pharmaceutical composition comprising a sustained release formulation.

137. A process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting nonneuronal cells expressing a Y5 receptor, with the chemical compound under conditions suitable for activation of the Y5 receptor, and measuring the binding of GTPγS to the cells in the presence and in the absence of the chemical compound, a change in the binding of GTPγS in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor.

138. A process for determining whether a chemical compound specifically binds to and activates a Y5 receptor, which comprises contacting a membrane fraction from a cell extract of nonneuronal cells expressing a Y5 receptor, with the chemical compound under conditions suitable for activation of the Y5 receptor, and measuring the binding of GTPγS to the membrane fraction in the presence and in the absence of the chemical compound, a change in the binding of GTPγS in the presence of the chemical compound indicating that the chemical compound activates the Y5 receptor.

139. A process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting nonneuronal cells expressing a Y5 receptor, with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, under conditions suitable for activation of the Y5 receptor, and measuring binding of GTPγS to the cells in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in GTPγS binding in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of a Y5 receptor.

140. A process for determining whether a chemical compound specifically binds to and inhibits activation of a Y5 receptor, which comprises separately contacting a membrane fraction from a cell extract of nonneuronal cells expressing a Y5 receptor, with both the chemical compound and a second chemical compound known to activate the Y5 receptor, and with only the second chemical compound, and measuring binding of the GTPγS to the membrane fraction in the presence of only the second chemical compound and in the presence of both the chemical compound and the second chemical compound, a smaller change in GTPγS binding in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemcial compound indicating that the chemical compound inhibits activation of a Y5 receptor.

141. The method of claim 137 or 138, wherein the change

is an increase in GTPγS binding.

142. The method of claim 139 or 140, wherein the change is a smaller increase in GTPγS binding in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

143. A method of decreasing feeding behavior of a subject which comprises administering to the subject a compound which is a galanin receptor antagonist and a compound which is a Y5 receptor antagonist, wherein the amount of the antagonists is effective to decrease the feeding behavior of the subject.

144. The method of claim 143, wherein the galanin receptor antagonist and the Y5 receptor antagonist are administered in combination.

145. The method of claim 143, wherein the galanin receptor antagonist and the Y5 receptor antagonist are administered once.

146. The method of claim 143, wherein the galanin receptor antagonist and the Y5 receptor antagonist are administered separately.

147. The method of claim 146, wherein the galanin receptor antagonist and the Y5 receptor antagonist are administered once.

148. The method of claim 146, wherein the galanin receptor antagonist is administered for about 1 week to about 2 weeks.

149. The method of claim 146, wherein the Y5 receptor antagonist is administered for about 1 week to about

2 weeks.

150. The method of claim 146, wherein the galanin receptor antagonist and the Y5 receptor antagonist are administerd alternately.

151. The method of claim 150, wherein the galanin receptor antagonist and the Y5 receptor antagonist are administered repeatedly.

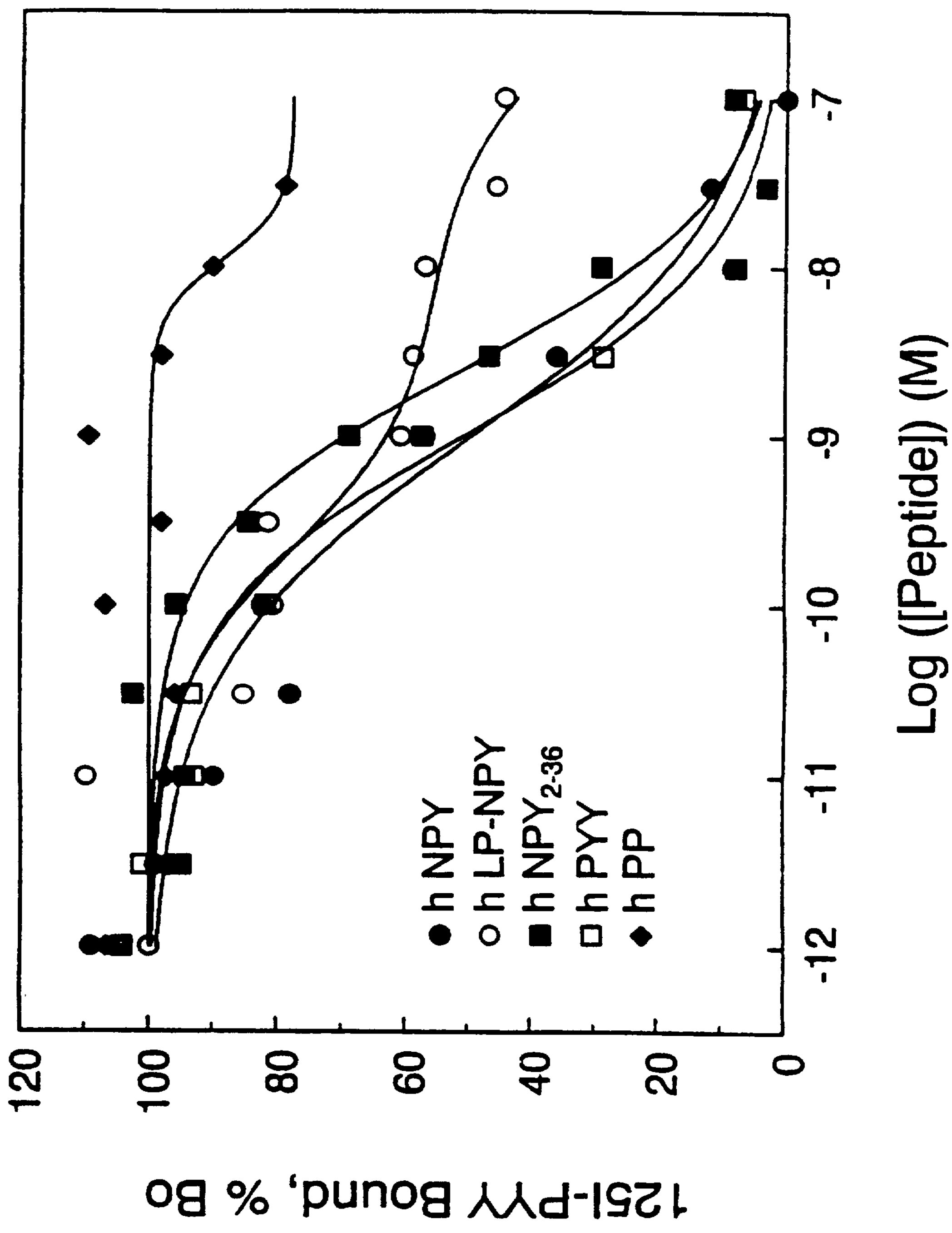
152. The method of claim 150 or 151, wherein the galanin receptor antagonist is administered for about 1 week to about 2 weeks.

153. The method of claim 143, 144, 145, 146, 147, 148, 149, 150, 151, or 152, wherein the galanin receptor is a GALR2 receptor.

154. The method of claim 143, 144, 145, 146, 147, 148, 149, 150, 151, or 152, wherein the galanin receptor is a GALR3 receptor.

155. The method of claim 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, or 154, wherein the compound is administered in a sustained release formulation.

FIGURE 1



h NPY
h LP-NPY
h NPY_{2-36}
h PYY
h PP
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
1251-PYY Bound, % Bo
Log ([Peptide]) (M)

FIGURE 2

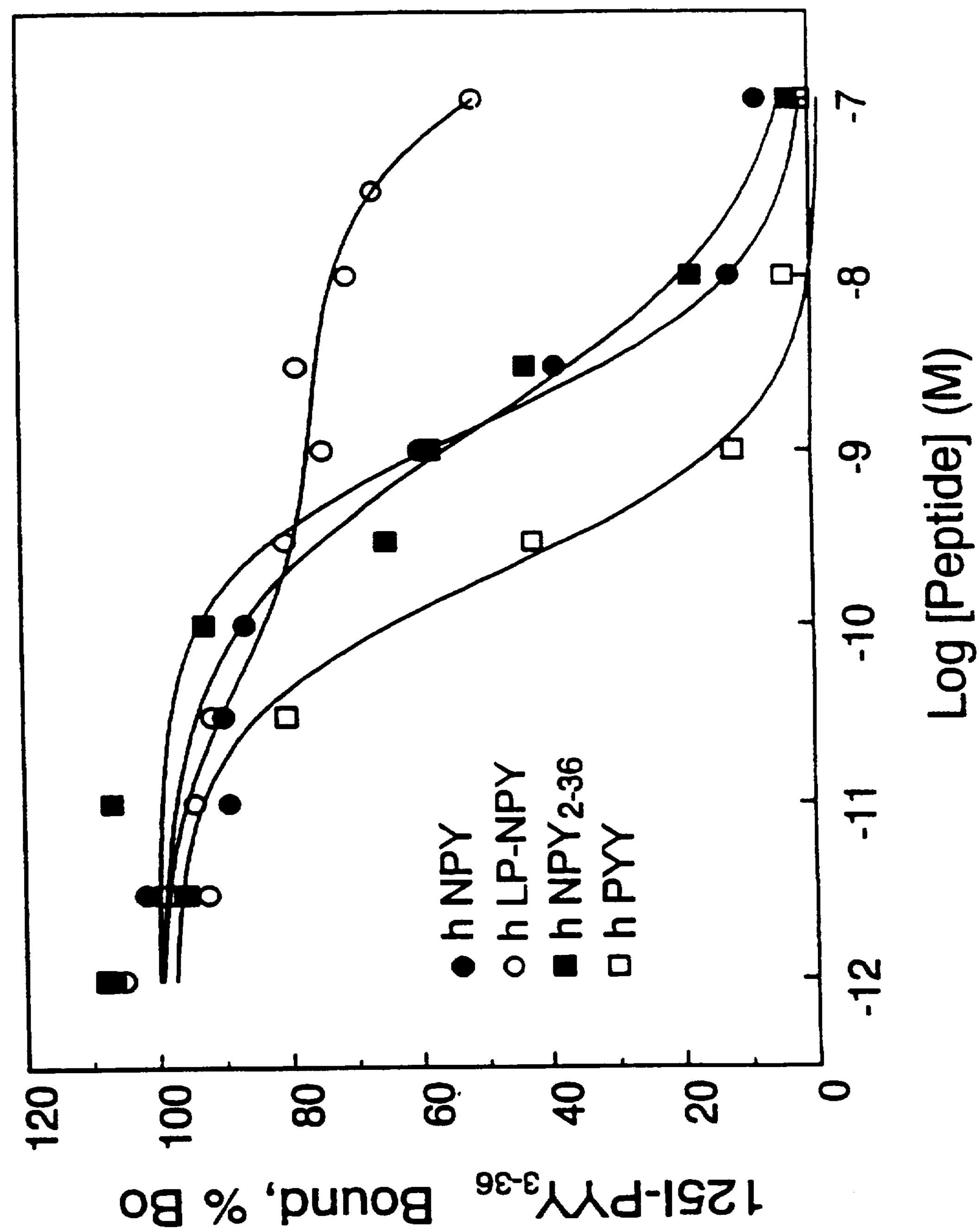

h NPY
h LP-NPY
h NPY2-36
h PYY
Log [Peptide] (M)
-12
-11
-10
-9
-8
-7
1251-PYY3-36 Bound, % Bo
0
20
40
60
80
100
120

FIGURE 3

```
   1  TTAGTTTTGTTCTGAGAACGTTAGAGTTATAGTACCGTGCGATCGTTCTTCAAGCTGCTA   60
  61  ATGGACGTCCTCTTCTTCCACCAGGATTCTAGTATGGAGTTTAAGCTTGAGGAGCATTTT  120
 121  AACAAGACATTTGTCACAGAGAACAATACAGCTGCTGCTCGGAATGCAGCCTTCCCTGCC  180
 181  TGGGAGGACTACAGAGGCAGCGTAGACGATTTACAATACTTTCTGATTGGGCTCTATACA  240
 241  TTCGTAAGTCTTCTTGGCTTTATGGGCAATCTACTTATTTTAATGGCTGTTATGAAAAAG  300
 301  CGCAATCAGAAGACTACAGTGAACTTTCTCATAGGCAACCTGGCCTTCTCCGACATCTTG  360
 361  GTCGTCCTGTTTTGCTCCCCTTTCACCCTGACCTCTGTCTTGTTGGATCAGTGGATGTTT  420
 421  GGCAAAGCCATGTGCCATATCATGCCGTTCCTTCAATGTGTGTCAGTTCTGGTTTCAACT  480
 481  CTGATTTTAATATCAATTGCCATTGTCAGGTATCATATGATAAAGCACCCTATTTCTAAC  540
 541  AATTTAACGGCAAACCATGGCTACTTCCTGATAGCTACTGTCTGGACACTGGGCTTTGCC  600
 601  ATCTGTTCTCCCCTCCCAGTGTTTCACAGTCTTGTGGAACTTAAGGAGACCTTTGGCTCA  660
 661  GCACTGCTGAGTAGCAAATATCTCTGTGTTGAGTCATGGCCCTCTGATTCATACAGAATT  720
 721  GCTTTCACAATCTCTTTATTGCTAGTGCAGTATATCCTGCCTCTAGTATGTTTAACGGTA  780
 781  AGTCATACCAGCGTCTGCCGAAGCATAAGCTGTGGATTGTCCCACAAAGAAAACAGACTC  840
 841  GAAGAAAATGAGATGATCAACTTAACCCTACAGCCATCCAAAAAGAGCAGGAACCAGGCA  900
 901  AAAACCCCCAGCACTCAAAAGTGGAGCTACTCATTCATCAGAAAGCACAGAAGGAGGTAC  960
 961  AGCAAGAAGACGGCCTGTGTCTTACCCGCCCCAGCAGGACCTTCCCAGGGGAAGCACCTA 1020
1021  GCCGTTCCAGAAAATCCAGCCTCCGTCCGTAGCCAGCTGTCGCCATCCAGTAAGGTCATT 1080
1081  CCAGGGGTCCCAATCTGCTTTGAGGTGAAACCTGAAGAAAGCTCAGATGCTCATGAGATG 1140
1141  AGAGTCAAGCGTTCCATCACTAGAATAAAAAAGAGATCTCGAAGTGTTTTCTACAGACTG 1200
1201  ACCATACTGATACTCGTGTTCGCCGTTAGCTGGATGCCACTCCACGTCTTCCACGTGGTG 1260
1261  ACTGACTTCAATGATAACTTGATTTCCAATAGGCATTTCAAGCTGGTATACTGCATCTGT 1320
1321  CACTTGTTAGGCATGATGTCCTGTTGTCTAAATCCGATCCTATATGGTTTCCTTAATAAT 1380
1381  GGTATCAAAGCAGACTTGAGAGCCCTTATCCACTGCCTACACATGTCATGATTCTCTCTG 1440
1441  TGCACCAAAGAGAGAAGAAACGTGGTAATTGACACATAATTTATACAGAAGTATTCTGGAT 1501
```

FIGURE 4

1	M	D	V	L	F	F	H	Q	D	S	S	M	E	F	K	L	E	E	H	F	20
21	N	K	T	F	V	T	E	N	N	T	A	A	A	R	N	A	A	F	P	A	40
41	W	E	D	Y	R	G	S	V	D	D	L	Q	Y	F	L	I	G	L	Y	T	60
61	F	V	S	L	L	G	F	M	G	N	L	L	I	L	M	A	V	M	K	K	80
81	R	N	Q	K	T	T	V	N	F	L	I	G	N	L	A	F	S	D	I	L	100
101	V	V	L	F	C	S	P	F	T	L	T	S	V	L	L	D	Q	W	M	F	120
121	G	K	A	M	C	H	I	M	P	F	L	Q	C	V	S	V	L	V	S	T	140
141	L	I	L	I	S	I	A	I	V	R	Y	H	M	I	K	H	P	I	S	N	160
161	N	L	T	A	N	H	G	Y	F	L	I	A	T	V	W	T	L	G	F	A	180
181	I	C	S	P	L	P	V	F	H	S	L	V	E	L	K	E	T	F	G	S	200
201	A	L	L	S	S	K	Y	L	C	V	E	S	W	P	S	D	S	Y	R	I	220
221	A	F	T	I	S	L	L	L	V	Q	Y	I	L	P	L	V	C	L	T	V	240
241	S	H	T	S	V	C	R	S	I	S	C	G	L	S	H	K	E	N	R	L	260
261	E	E	N	E	M	I	N	L	T	L	Q	P	S	K	K	S	R	N	Q	A	280
281	K	T	P	S	T	Q	K	W	S	Y	S	F	I	R	K	H	R	R	R	Y	300
301	S	K	K	T	A	C	V	L	P	A	P	A	G	P	S	Q	G	K	H	L	320
321	A	V	P	E	N	P	A	S	V	R	S	Q	L	S	P	S	S	K	V	I	340
341	P	G	V	P	I	C	F	E	V	K	P	E	E	S	S	D	A	H	E	M	360
361	R	V	K	R	S	I	T	R	I	K	K	R	S	R	S	V	F	Y	R	L	380
381	T	I	L	I	L	V	F	A	V	S	W	M	P	L	H	V	F	H	V	V	400
401	T	D	F	N	D	N	L	I	S	N	R	H	F	K	L	V	Y	C	I	C	420
421	H	L	L	G	M	M	S	C	C	L	N	P	I	L	Y	G	F	L	N	N	440
441	G	I	K	A	D	L	R	A	L	I	H	C	L	H	M	S					456

FIGURE 5

```
   1  GTTTCCCTCTGAATAGATTAATTTAAAGTAGTCATGTAATGTTTTTTTGGTTGCTGACAA    60
  61  ATGTCTTTTTATTCCAAGCAGGACTATAATATGGATTTAGAGCTCGACGAGTATTATAAC   120
 121  AAGACACTTGCCACAGAGAATAATACTGCTGCCACTCGGAATTCTGATTTCCCAGTCTGG   180
 181  GATGACTATAAAAGCAGTGTAGATGACTTACAGTATTTTCTGATTGGGCTCTATACATTT   240
 241  GTAAGTCTTCTTGGCTTTATGGGGAATCTACTTATTTTAATGGCTCTCATGAAAAAGCGT   300
 301  AATCAGAAGACTACGGTAAACTTCCTCATAGGCAATCTGGCCTTTTCTGATATCTTGGTT   360
 361  GTGCTGTTTTGCTCACCTTTCACACTGACGTCTGTCTTGCTGGATCAGTGGATGTTTGGC   420
 421  AAAGTCATGTGCCATATTATGCCTTTTCTTCAATGTGTGTCAGTTTTGGTTTCAACTTTA   480
 481  ATTTTAATATCAATTGCCATTGTCAGGTATCATATGATAAAACATCCCATATCTAATAAT   540
 541  TTAACAGCAAACCATGGCTACTTTCTGATAGCTACTGTCTGGACACTAGGTTTTGCCATC   600
 601  TGTTCTCCCCTTCCAGTGTTTCACAGTCTTGTGGAACTTCAAGAAACATTTGGTTCAGCA   660
 661  TTGCTGAGCAGCAGGTATTTATGTGTTGAGTCATGGCCATCTGATTCATACAGAATTGCC   720
 721  TTTACTATCTCTTTATTGCTAGTTCAGTATATTCTGCCCTTAGTTTGTCTTACTGTAAGT   780
 781  CATACAAGTGTCTGCAGAAGTATAAGCTGTGGATTGTCCAACAAAGAAAACAGACTTGAA   840
 841  GAAAATGAGATGATCAACTTAACTCTTCATCCATCCAAAAAGAGTGGGCCTCAGGTGAAA   900
 901  CTCTCTGGCAGCCATAAATGGAGTTATTCATTCATCAAAAAACACAGAAGAAGATATAGC   960
 961  AAGAAGACAGCATGTGTGTTACCTGCTCCAGAAAGACCTTCTCAAGAGAACCACTCCAGA  1020
1021  ATACTTCCAGAAAACTTTGGCTCTGTAAGAAGTCAGCTCTCTTCATCCAGTAAGTTCATA  1080
1081  CCAGGGGTCCCCACTTGCTTTGAGATAAAACCTGAAGAAAATTCAGATGTTCATGAATTG  1140
1141  AGAGTAAAACGTTCTGTTACAAGAATAAAAAAGAGATCTCGAAGTGTTTTCTACAGACTG  1200
1201  ACCATACTGATATTAGTATTTGCTGTTAGTTGGATGCCACTACACCTTTTCCATGTGGTA  1260
1261  ACTGATTTTAATGACAATCTTATTTCAAATAGGCATTTCAAGTTGGTGTATTGCATTTGT  1320
1321  CATTTGTTGGGCATGATGTCCTGTTGTCTTAATCCAATTCTATATGGGTTTCTTAATAAT  1380
1381  GGGATTAAAGCTGATTTAGTGTCCCTTATACACTGTCTTCATATGTAATAATTCTCACTG  1440
1441  TTTACCAAGGAAAGAAC                                             1457
```

FIGURE 6

1	M	S	F	Y	S	K	Q	D	Y	N	M	D	L	E	L	D	E	Y	Y	N	20
21	K	T	L	A	T	E	N	N	T	A	A	T	R	N	S	D	F	P	V	W	40
41	D	D	Y	K	S	S	V	D	D	L	Q	Y	F	L	I	G	L	Y	T	F	60
61	V	S	L	L	G	F	M	G	N	L	L	I	L	M	A	L	M	K	K	R	80
81	N	Q	K	T	T	V	N	F	L	I	G	N	L	A	F	S	D	I	L	V	100
101	V	L	F	C	S	P	F	T	L	T	S	V	L	L	D	Q	W	M	F	G	120
121	K	V	M	C	H	I	M	P	F	L	Q	C	V	S	V	L	V	S	T	L	140
141	I	L	I	S	I	A	I	V	R	Y	H	M	I	K	H	P	I	S	N	N	160
161	L	T	A	N	H	G	Y	F	L	I	A	T	V	W	T	L	G	F	A	I	180
181	C	S	P	L	P	V	F	H	S	L	V	E	L	Q	E	T	F	G	S	A	200
201	L	L	S	S	R	Y	L	C	V	E	S	W	P	S	D	S	Y	R	I	A	220
221	F	T	I	S	L	L	L	V	Q	Y	I	L	P	L	V	C	L	T	V	S	240
241	H	T	S	V	C	R	S	I	S	C	G	L	S	N	K	E	N	R	L	E	260
261	E	N	E	M	I	N	L	T	L	H	P	S	K	K	S	G	P	Q	V	K	280
281	L	S	G	S	H	K	W	S	Y	S	F	I	K	K	H	R	R	R	Y	S	300
301	K	K	T	A	C	V	L	P	A	P	E	R	P	S	Q	E	N	H	S	R	320
321	I	L	P	E	N	F	G	S	V	R	S	Q	L	S	S	S	S	K	F	I	340
341	P	G	V	P	T	C	F	E	I	K	P	E	E	N	S	D	V	H	E	L	360
361	R	V	K	R	S	V	T	R	I	K	K	R	S	R	S	V	F	Y	R	L	380
381	T	I	L	I	L	V	F	A	V	S	W	M	P	L	H	L	F	H	V	V	400
401	T	D	F	N	D	N	L	I	S	N	R	H	F	K	L	V	Y	C	I	C	420
421	H	L	L	G	M	M	S	C	C	L	N	P	I	L	Y	G	F	L	N	N	440
441	G	I	K	A	D	L	V	S	L	I	H	C	L	H	M						455

FIGURE 7A

FIGURE 7A
FIGURE 7B
FIGURE 7C
FIGURE 7D
FIGURE 7E

```
  1 ATGGACGTCCTCTTCTTCC.ACCAGGATTCTAGTATGGAGTTTAAGCTTG  50

  1 ....ATGTCTTTTTATTCCAAGCAGGACTATAATATGGATTTAGAGCTCG  46

 51 AGGAGCATTTTAACAAGACATTTGTCACAGAGAACAATACAGCTGCTGCT 100

 47 ACGAGTATTATAACAAGACACTTGCCACAGAGAATAATACTGCTGCCACT  96

101 CGGAATGCAGCCTTCCCTGCCTGGGAGGACTACAGAGGCAGCGTAGACGA 150

 97 CGGAATTCTGATTTCCCAGTCTGGGATGACTATAAAAGCAGTGTAGATGA 146

151 TTTACAATACTTTCTGATTGGGCTCTATACATTCGTAAGTCTTCTTGGCT 200

147 CTTACAGTATTTTCTGATTGGGCTCTATACATTTGTAAGTCTTCTTGGCT 196

201 TTATGGGCAATCTACTTATTTTAATGGCTGTTATGAAAAAGCGCAATCAG 250

197 TTATGGGGAATCTACTTATTTTAATGGCTCTCATGAAAAAGCGTAATCAG 246
```

FIGURE 7B

```
              .         .         .         .         .
251 AAGACTACAGTGAACTTTCTCATAGGCAACCTGGCCTTCTCCGACATCTT 300
    |||||||| || ||||| ||||||||||| |||||||| || || |||||
247 AAGACTACGGTAAACTTCCTCATAGGCAATCTGGCCTTTTCTGATATCTT 296
              .         .         .         .         .
301 GGTCGTCCTGTTTTGCTCCCCTTTCACCCTGACCTCTGTCTTGTTGGATC 350
    ||| || ||||||||||| |||||||| ||||| ||||||||| ||||||
297 GGTTGTGCTGTTTTGCTCACCTTTCACACTGACGTCTGTCTTGCTGGATC 346
              .         .         .         .         .
351 AGTGGATGTTTGGCAAAGCCATGTGCCATATCATGCCGTTCCTTCAATGT 400
    |||||||||||||||||| |||||||||||| ||||| || |||||||||
347 AGTGGATGTTTGGCAAAGTCATGTGCCATATTATGCCTTTTCTTCAATGT 396
              .         .         .         .         .
401 GTGTCAGTTCTGGTTTCAACTCTGATTTTAATATCAATTGCCATTGTCAG 450
    ||||||||| ||||||||||| | ||||||||||||||||||||||||||
397 GTGTCAGTTTTGGTTTCAACTTTAATTTTAATATCAATTGCCATTGTCAG 446
              .         .         .         .         .
451 GTATCATATGATAAAGCACCCTATTTCTAACAATTTAACGGCAAACCATG 500
    ||||||||||||||| || || || ||||| |||||||| ||||||||||
447 GTATCATATGATAAAACATCCCATATCTAATAATTTAACAGCAAACCATG 496
              .         .         .         .         .
501 GCTACTTCCTGATAGCTACTGTCTGGACACTGGGCTTTGCCATCTGTTCT 550
    ||||||| ||||||||||||||||||||||| || |||||||||||||||
497 GCTACTTTCTGATAGCTACTGTCTGGACACTAGGTTTTGCCATCTGTTCT 546
```

FIGURE 7C

```
              .         .         .         .         .
551 CCCCTCCCAGTGTTTCACAGTCTTGTGGAACTTAAGGAGACCTTTGGCTC 600
    ||||| ||||||||||||||||||||||||||| | || || ||||| ||
547 CCCCTTCCAGTGTTTCACAGTCTTGTGGAACTTCAAGAAACATTTGGTTC 596
              .         .         .         .         .
601 AGCACTGCTGAGTAGCAAATATCTCTGTGTTGAGTCATGGCCCTCTGATT 650
    |||| ||||||| ||||  ||| | ||||||||||||||||| |||||||
597 AGCATTGCTGAGCAGCAGGTATTTATGTGTTGAGTCATGGCCATCTGATT 646
              .         .         .         .         .
651 CATACAGAATTGCTTTCACAATCTCTTTATTGCTAGTGCAGTATATCCTG 700
    ||||||||||||| || || ||||||||||||||||| |||||||| |||
647 CATACAGAATTGCCTTTACTATCTCTTTATTGCTAGTTCAGTATATTCTG 696
              .         .         .         .         .
701 CCTCTAGTATGTTTAACGGTAAGTCATACCAGCGTCTGCCGAAGCATAAG 750
    ||  |||| ||| | || ||||||||||| || |||||| |||| |||||
697 CCCTTAGTTTGTCTTACTGTAAGTCATACAAGTGTCTGCAGAAGTATAAG 746
              .         .         .         .         .
751 CTGTGGATTGTCCCACAAAGAAAACAGACTCGAAGAAAATGAGATGATCA 800
    ||||||||||||| |||||||||||||||| |||||||||||||||||||
747 CTGTGGATTGTCCAACAAAGAAAACAGACTTGAAGAAAATGAGATGATCA 796
              .         .         .         .         .
801 ACTTAACCCTACAGCCATCCAAAAAGAGCAGGAACCAGGCAAAAACCCCC 850
    ||||||| || || ||||||||||||||  ||   ||||  |||  | | 
797 ACTTAACTCTTCATCCATCCAAAAAGAGTGGGCCTCAGGTGAAACTCTCT 846
```

FIGURE 7D

```
 851 AGCACTCAAAAGTGGAGCTACTCATTCATCAGAAAGCACAGAAGGAGGTA  900

 847 GGCAGCCATAAATGGAGTTATTCATTCATCAAAAAACACAGAAGAAGATA  896

 901 CAGCAAGAAGACGGCCTGTGTCTTACCCGCCCCAGCAGGACCTTCCCAGG  950

 897 TAGCAAGAAGACAGCATGTGTGTTACCTGCTCCAGAAAGACCTTCTCAAG  946

 951 GGAAGCA...CCTAGCCGTTCCAGAAAATCCAGCCTCCGTCCGTAGCCAG 1000

 947 AGAACCACTCCAGAATACTTCCAGAAAACTTTGGCTCTGTAAGAAGTCAG  996

1001 CTGTCGCCATCCAGTAAGGTCATTCCAGGGGTCCCAATCTGCTTTGAGGT 1050

 997 CTCTCTTCATCCAGTAAGTTCATACCAGGGGTCCCCACTTGCTTTGAGAT 1046

1051 GAAACCTGAAGAAAGCTCAGATGCTCATGAGATGAGAGTCAAGCGTTCCA 1100

1047 AAAACCTGAAGAAAATTCAGATGTTCATGAATTGAGAGTAAAACGTTCTG 1096

1101 TCACTAGAATAAAAAAGAGATCTCGAAGTGTTTTCTACAGACTGACCATA 1150

1097 TTACAAGAATAAAAAAGAGATCTCGAAGTGTTTTCTACAGACTGACCATA 1146
```

FIGURE 7E

```
               .         .         .         .         .
1151 CTGATACTCGTGTTCGCCGTTAGCTGGATGCCACTCCACGTCTTCCACGT 1200
     |||||| | || || || ||||| ||||||||||| ||| | ||||| ||
1147 CTGATATTAGTATTTGCTGTTAGTTGGATGCCACTACACCTTTTCCATGT 1196

               .         .         .         .         .
1201 GGTGACTGACTTCAATGATAACTTGATTTCCAATAGGCATTTCAAGCTGG 1250
     ||| ||||| || ||||| ||  | ||||| ||||||||||||||| |||
1197 GGTAACTGATTTTAATGACAATCTTATTTCAAATAGGCATTTCAAGTTGG 1246

               .         .         .         .         .
1251 TATACTGCATCTGTCACTTGTTAGGCATGATGTCCTGTTGTCTAAATCCG 1300
     | || ||||| ||||| ||||| |||||||||||||||||||| ||||| 
1247 TGTATTGCATTTGTCATTTGTTGGGCATGATGTCCTGTTGTCTTAATCCA 1296

               .         .         .         .         .
1301 ATCCTATATGGTTTCCTTAATAATGGTATCAAAGCAGACTTGAGAGCCCT 1350
     || |||||||| || ||||||||||| || ||||| || ||     ||||
1297 ATTCTATATGGGTTTCTTAATAATGGGATTAAAGCTGATTTAGTGTCCCT 1346

               .         .
1351 TATCCACTGCCTACACATGTCA 1372
     ||| ||||| || || |||
1347 TATACACTGTCTTCATATG... 1365
```

FIGURE 7F

FIGURE 7F
FIGURE 7G

```
                  .         .         .         .         .
  1  MDVLFFHQDSSMEFKLEEHFNKTFVTENNTAAARNAAFPAWEDYRGSVDD   50
        ::  .||  .|::.|:|.:|||:.|||||||.||..||.|:||::||||
  1  .MSFYSKQDYNMDLELDEYYNKTLATENNTAATRNSDFPVWDDYKSSVDD   49
      ┌─────────── I ─────────────┐ .      ┌───── II ─
 51  LQYFLIGLYTFVSLLGFMGNLLILMAVMKKRNQKTTVNFLIGNLAFSDIL  100
     ||||||||||||||||||||||||||:|||||||||||||||||||||||
 50  LQYFLIGLYTFVSLLGFMGNLLILMALMKKRNQKTTVNFLIGNLAFSDIL   99
     ──────────┐         .       ┌──────── III ────────┐ .
101  VVLFCSPFTLTSVLLDQWMFGKAMCHIMPFLQCVSVLVSTLILISIAIVR  150
     ||||||||||||||||||||||.|||||||||||||||||||||||||||
100  VVLFCSPFTLTSVLLDQWMFGKVMCHIMPFLQCVSVLVSTLILISIAIVR  149
              .         ┌─────── IV ────────┐ .         .
151  YHMIKHPISNNLTANHGYFLIATVWTLGFAICSPLPVFHSLVELKETFGS  200
     ||||||||||||||||||||||||||||||||||||||||||||.|||||
150  YHMIKHPISNNLTANHGYFLIATVWTLGFAICSPLPVFHSLVELQETFGS  199
```

FIGURE 7G

```
                                  V
201 ALLSSKYLCVESWPSDSYRIAFTISLLLVQYILPLVCLTVSHTSVCRSIS 250
    |||||:||||||||||||||||||||||||||||||||||||||||||||
200 ALLSSRYLCVESWPSDSYRIAFTISLLLVQYILPLVCLTVSHTSVCRSIS 249

251 CGLSHKENRLEENEMINLTLQPSKKSRNQAKTPSTQKWSYSFIRKHRRRY 300
    ||||:|||||||||||||||:|||||  |.| .:.:|||||||:||||||
250 CGLSNKENRLEENEMINLTLHPSKKSGPQVKLSGSHKWSYSFIKKHRRRY 299

301 SKKTACVLPAPAGPSQGKHLAV.PENPASVRSQLSPSSKVIPGVPICFEV 349
    |||||||||||. |||:.|  :  ||| :|||||||.|||.|||||.|||:
300 SKKTACVLPAPERPSQENHSRILPENFGSVRSQLSSSSKFIPGVPTCFEI 349
                                          VI
350 KPEESSDAHEMRVKRSITRIKKRSRSVFYRLTILILVFAVSWMPLHVFHV 399
    ||||.||.||:|||||:|||||||||||||||||||||||||||||:|||
350 KPEENSDVHELRVKRSVTRIKKRSRSVFYRLTILILVFAVSWMPLHLFHV 399
                          VII
400 VTDFNDNLISNRHFKLVYCICHLLGMMSCCLNPILYGFLNNGIKADLRAL 449
    ||||||||||||||||||||||||||||||||||||||||||||||| .|
400 VTDFNDNLISNRHFKLVYCICHLLGMMSCCLNPILYGFLNNGIKADLVSL 449

450 IHCLHMS  456
    ||||||
450 IHCLHM.  455
```

FIGURE 8A

FIGURE 8A
FIGURE 8B
FIGURE 8C

```
                                                   II
Y5h  MSFYSKQDYNMDLELDEYYNKTLATENNTAATRNSDFPVWDDYKSSVDDL   50
Y1h             MN.STLFSQVENHSVHSNFSEKNAQLLA.FENDDCHLPL.AM   39
Y2h  MGPIGAEADENQTVEEMKVEQYGPQTTPRGELVPDPEPELIDSTKLI.EV   49
Y4h             MNTSHLLALLLPKSPQGENRSKPLGTPY.NFSEHCQDSV.DV   40

                  I                                II
Y5h  QYFLIGLYTFVSLLGFMGNLLILMALMKKRNQKTTVNFLIGNLAFSDILV  100
Y1h  IFTLALAYGAVIILGVSGNLALIIIILKQKEMRNVTNILIVNLSFSDLLV   89
Y2h  QVVLIILAYCSIILLGVIGNSLVIHVVIKFKSMRTVTNFFIANLAVADLLV  99
Y4h  MVFIVTSYSIETVVGVLGNLCLMCVTVRQKEKANVTNLLIANLAFSDFLM   90

                                       III
Y5h  VLFCSPFTLTSVLLDQWMFGKVMCHIMPFLQCVSVLVSTLILISIAIVRY  150
Y1h  AIMCLPLTFVYTLMDHWVFGEAMCKLNPFVQCVSITVSIFSLVLIAVERH  139
Y2h  NTLCLPFTLTYTLMGEWKMGPVLCHLVPYAQGLAVQVSTITLTVIALDRH  149
Y4h  CLLCQPLTAVYTIMDYWIFGETLCKMSAFIQCMSVTVSILSLVLVALERH  140

                              IV
Y5h  HMIKHPISNNLTANHGYFLIATVWTLGFAICSPLPVFHSLVELQETFGSA  200
Y1h  QLIINPRGWRPNNRHAYVGIAVIWVLAVASSLPFLIYQVMTDEPFQNVTL  189
Y2h  RCIVYHLESKISKRISFLIIGLAWGISALLASPLAIFREYSLIEIIPDFE  199
Y4h  QLIINPTGWKPSISQAYLGIVLIWVIACVLSLPFLANSILENVFHKNHSK  190
```

FIGURE 8B

```
                                    |----------------- V -----------------|
Y5h  LLS...SRYLCVESWPSD..S.YRIAFTISLLLVQYILPLVCLTVSHTSV   244
Y1h  D.AYK.DKYVCFDQFPSD..S.HRLSYTTLLLVLQYFGPLCFIFICYFKI   234
Y2h  IVA.......CTEKWPGEEKSIYGTVYSLSSLLILYVLPLGIISFSYTRI   242
Y4h  ALEFLADKVVCTESWP...LAHHRTIYTTFLLLFQYCLPLGFILVCYARI   237

Y5h  CRSISCGLSNKENRLEENEMINLTLHPSKKSGPQVKLSGSHKWSYSFIKK   294
Y1h  ................................................YI   236
Y2h  ............................................WSKLKN   248
Y4h  ................................................YR   239

Y5h  HRRRYSKKTACVLPAPERPSQENHSRILPENFGSVRSQLSSSSKFIPGVP   344
Y1h  RLKRRNNMMDKMRDNKYRSSE.............................   257
Y2h  HVSPGAANDHYHQRRQK.................................   265
Y4h  RLQRQGRVFHK.GTYSLRAGH.............................   259

                                    |------------- VI -----------
Y5h  TCFEIKPEENSDVHELRVKRSVTRIKKRSRSVFYRLTILILVFAVSWMPL   394
Y1h  ...............................TKRINIMLLSIVVAFAVCWLPL   279
Y2h  ..................................TTKMLVCVVVVFAVSWLPL   284
Y4h  ...............................MKQVNVVLVVMVVAFAVLWLPL   281
```

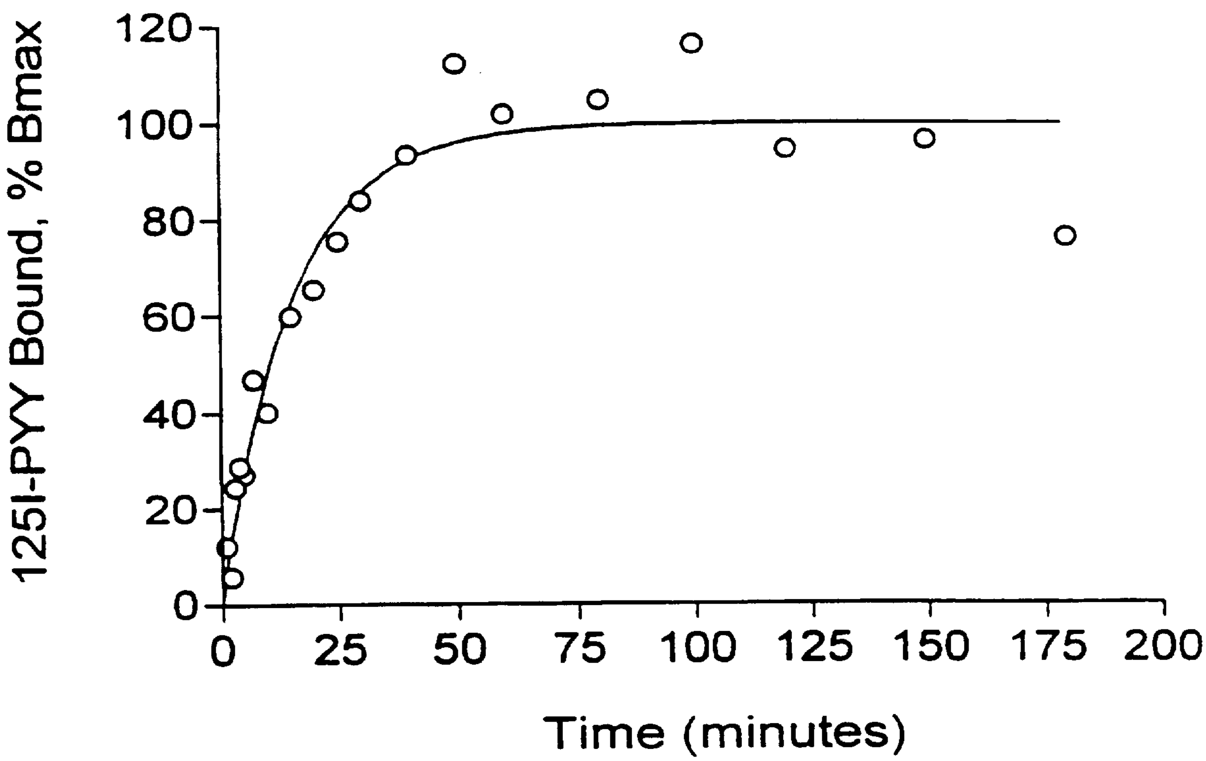
FIGURE 8C

```
                                                   VII
Y5h  H L F H V V T D F N D N L I S N R H F K L V Y C I C H L L G M M S C C L N P I L Y G F L N N G I K A  444
Y1h  T I F N T V F D W N H Q I I A T C N H N L L F L L C H L T A M I S T C V N P I F Y G F L N K N F Q R  329
Y2h  H A F Q L A V D I D S Q V L D L K E Y K L I F T V F H I I A M C S T F A N P L L Y G W M N S N Y R K  334
Y4h  H V F N S L E D W H H E A I P I C H G N L I F L V C H L L A M A S T C V N P F I Y G F L N T N F K K  331

Y5h  D L V S L I H . C L H M . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .  455
Y1h  D L Q F F F N F C D F R S R D D D Y E T I A M S T M H T D V S K T S L K Q A S P V A F K K I N N N D  379
Y2h  A F L S A F R . C E Q R L D A I H S E V S V T F K A K K N L E V R K N S G P N D S F T E A T N V . .  381
Y4h  E I K A L V L T C Q Q S A P L E E S E H L P L S T V H T E V S K G S L R L S G R S N P I . . . . . .  375

Y5h  . . . . .  455
Y1h  D N E K I  384
Y2h  . . . . .  365
Y4h  . . . . .  375
```

FIGURE 9



120
100
80
60
40
20
0
125I-PYY Bound, % Bmax
0
25
50
75
100
125
150
175
200
Time (minutes)

FIGURE 10

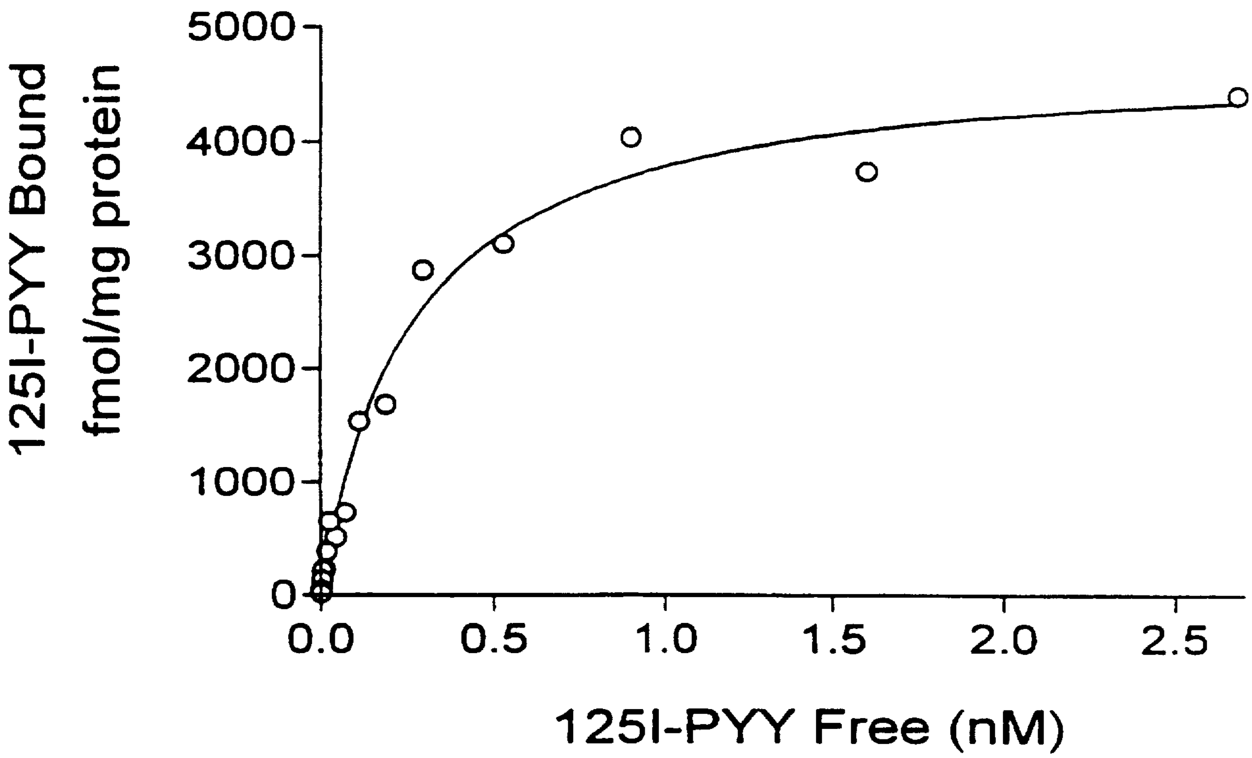

5000
4000
3000
2000
1000
0
125I-PYY Bound
fmol/mg protein
0.0
0.5
1.0
1.5
2.0
2.5
125I-PYY Free (nM)

FIGURE 11

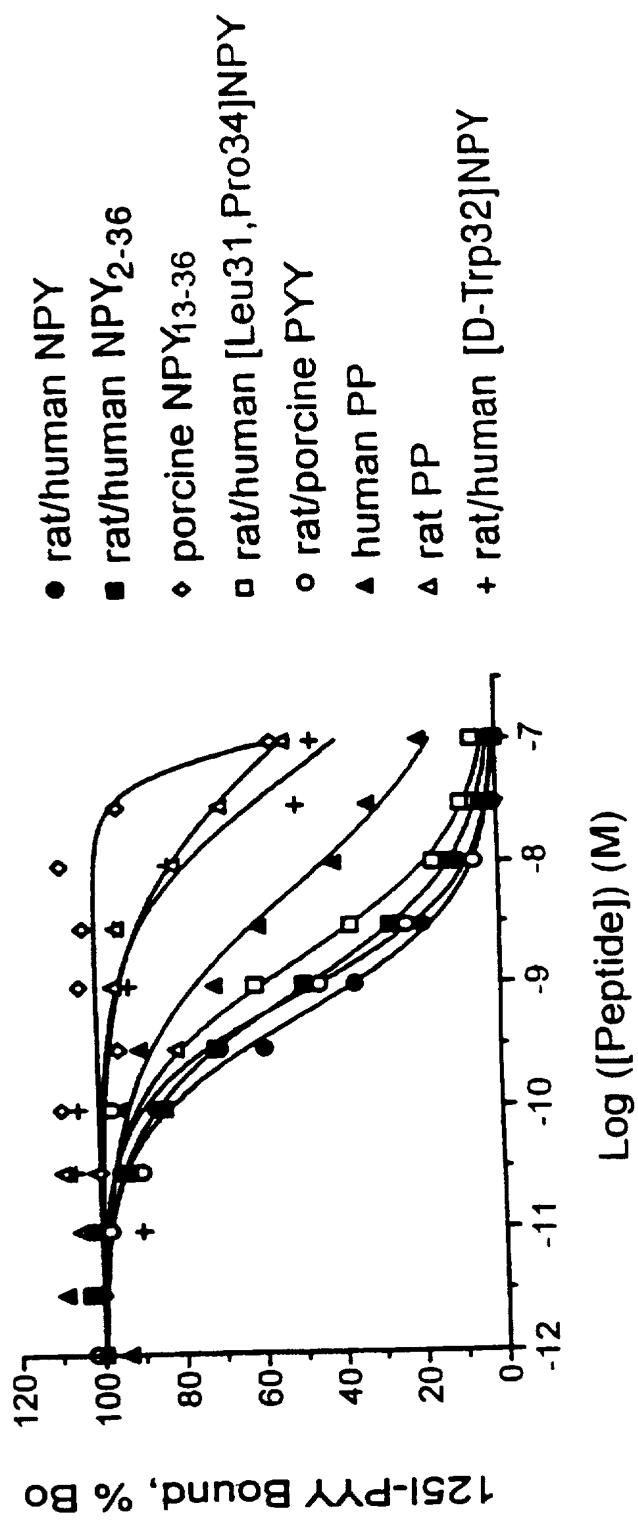

● rat/human NPY
■ rat/human NPY$_{2\text{-}36}$
◇ porcine NPY$_{13\text{-}36}$
□ rat/human [Leu31,Pro34]NPY
○ rat/porcine PYY
▲ human PP
△ rat PP
+ rat/human [D-Trp32]NPY
1251-PYY Bound, % Bo
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Log ([Peptide]) (M)

FIGURE 12

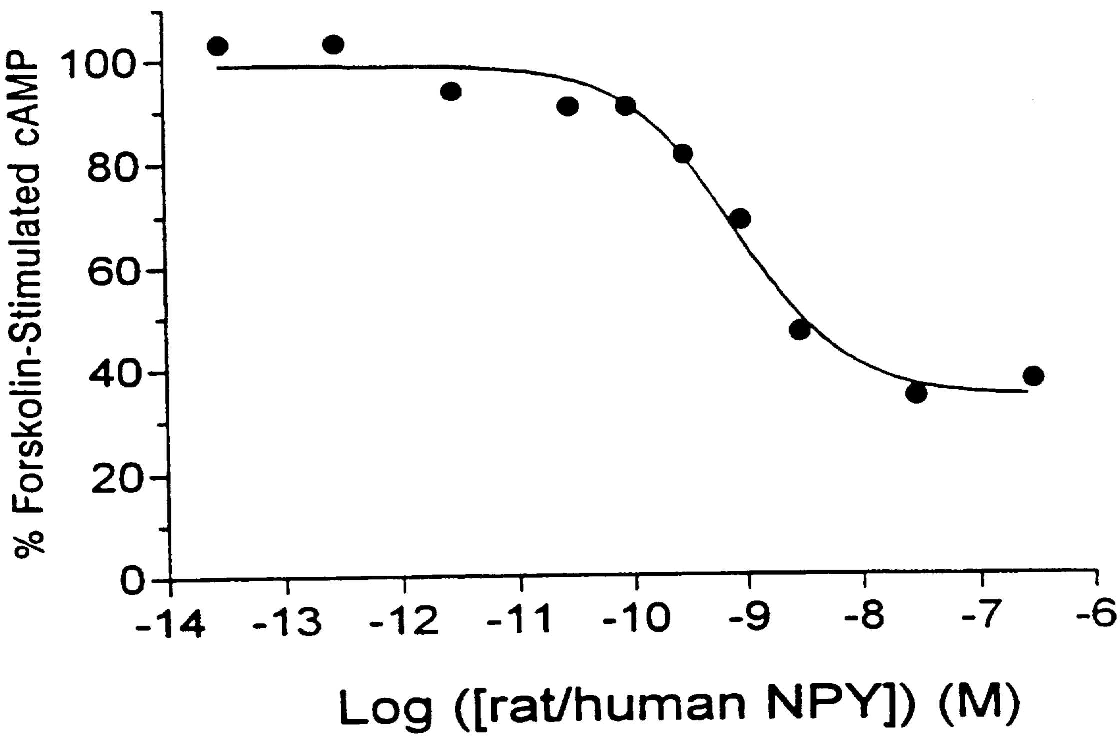

% Forskolin-Stimulated cAMP
100
80
60
40
20
0
-14
-13
-12
-11
-10
-9
-8
-7
-6
Log ([rat/human NPY]) (M)

FIGURE 13A

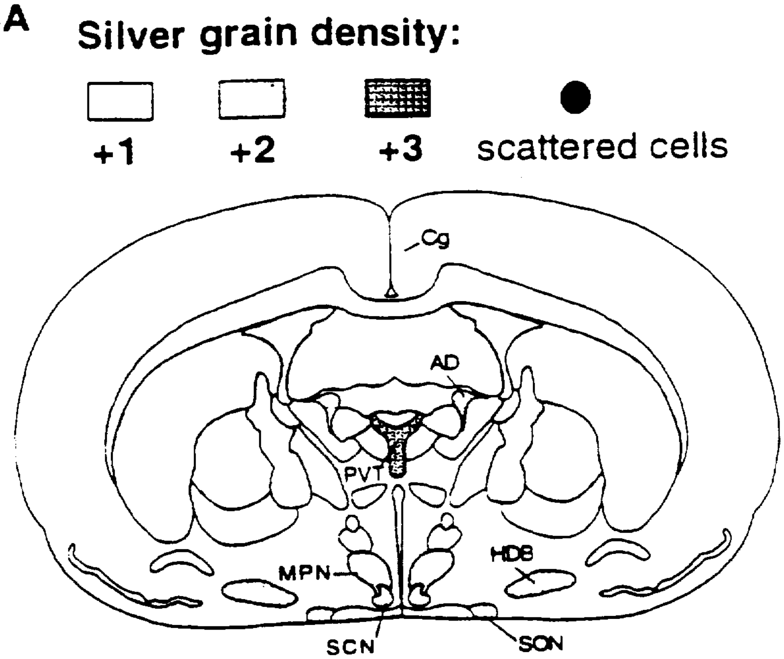


FIGURE 13B

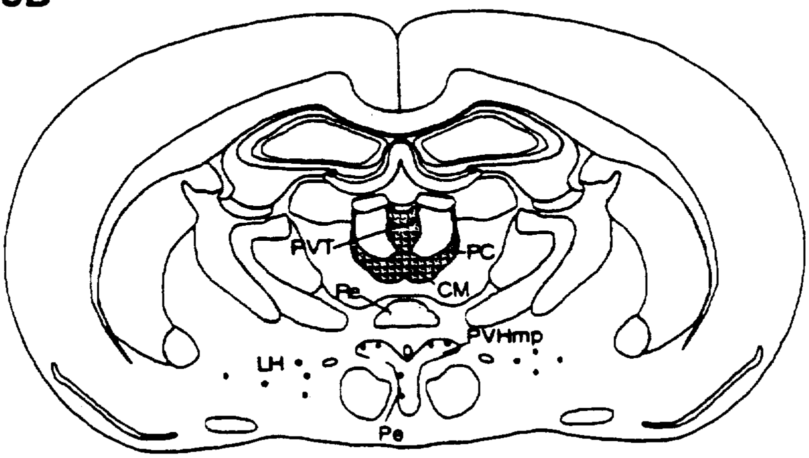


FIGURE 13C

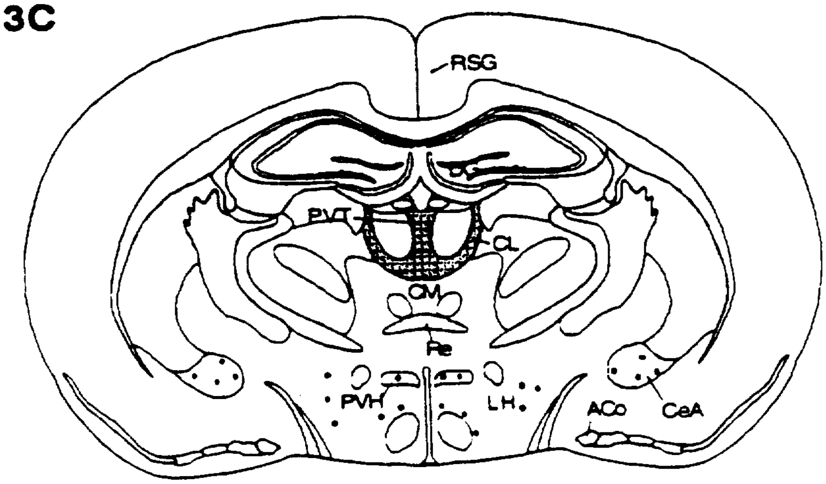


FIGURE 13D

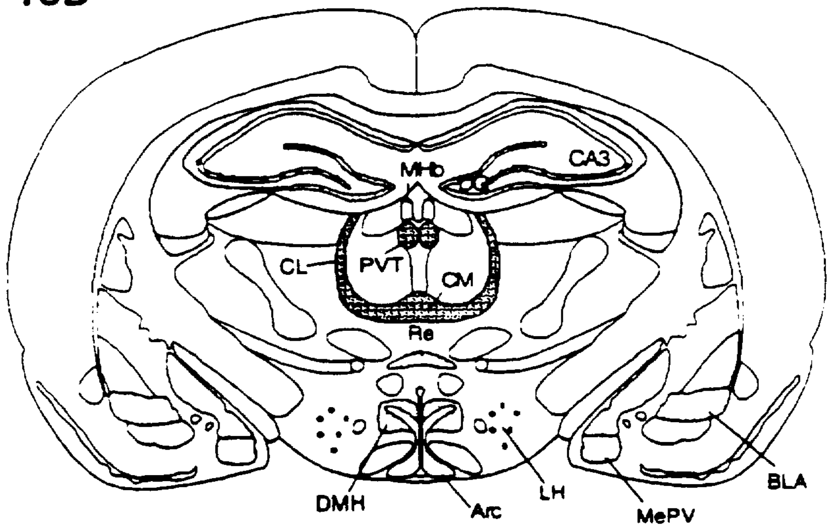


FIGURE 13E

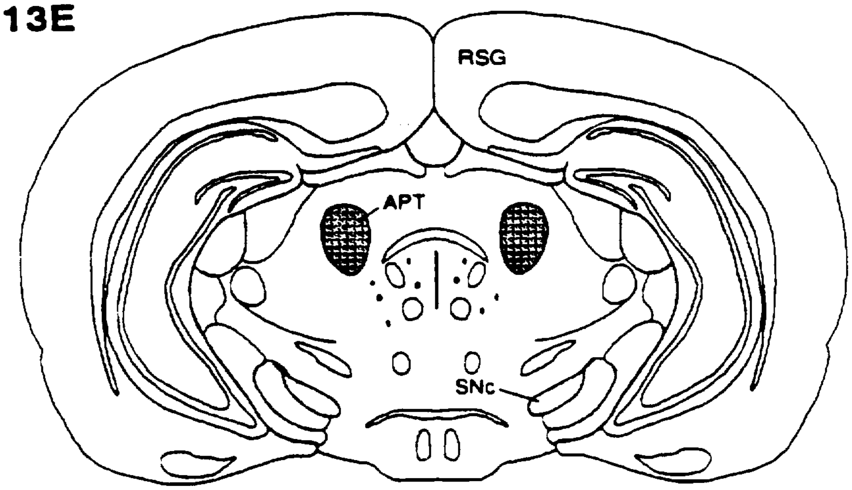


FIGURE 13F

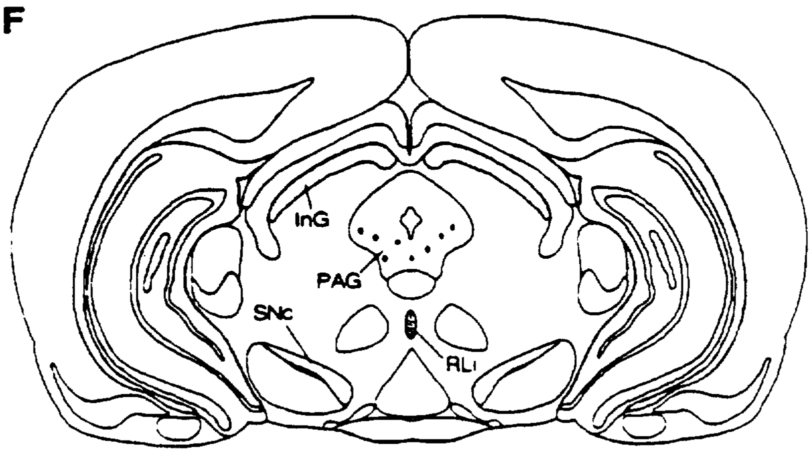


FIGURE 13G

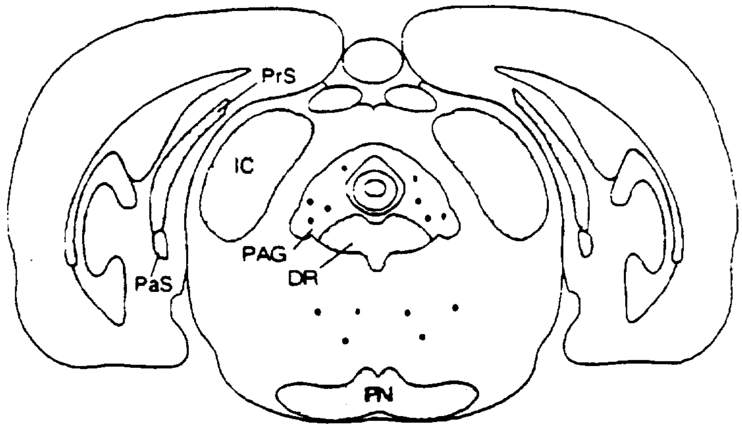


FIGURE 13H

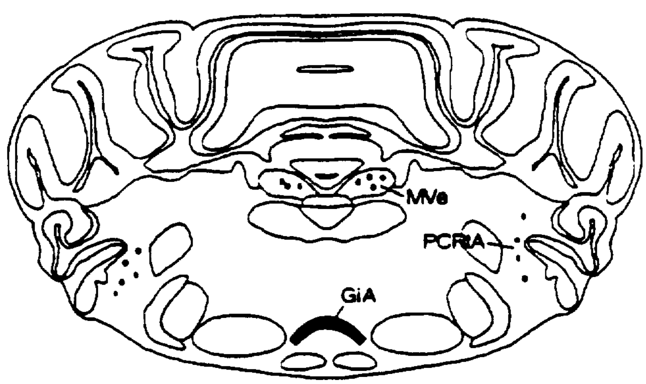


FIGURE 14

1	TCATGTGTCA	CATTATGCCT	TTTCTTCAAT	GTGTGTCAGT	TCTGGTTTCA	50
51	ACTTTAATTC	TAATATCAAT	TGCCATTGTC	AGGTATCATA	TGATCAAGCA	51
101	TCCTATATCT	AACAATTTAA	CAGCAAACCA	TGGCTACTTC	CTGATTGCTA	150
151	CTGTCTGGAC	ACTAGGTTTT	GCGATTTGTT	CTCCCCTTCC	AGTGTTTCAC	200
201	AGTCTGGTGG	AACTTCAGGA	AACATTTGAC	TCCGCATTGC	TGAGCAGCAG	250
251	GTATTTATGT	GTTGAGTCGT	GGCCATCTGA	TTCGTACAGA	ATCGCTTTTA	300
301	CTATCTCTTT	ATTGCTAGTC	CAGTATATTC	TTCCCTTGGT	GTGTCTAACT	350
351	GTGAGCCATA	CCAGTGTCTG	CAGGAGTATA	AGCTGCGGGT	TGTCCAACAA	400
401	AGAAAACAAA	CTGGAAGAAA	ACGAGATGAT	CAACTTAACT	CTTCAACCAT	450
451	TCAAAAAGAG	TGGGCCTCAG	GTGAAACTTT	CCAGCAGCCA	TAAATGGAGC	500
501	TATTCATTCA	TCAGAAAACA	CAGGAGAAGG	TACAGCAAGA	AGACGGCGTG	550
551	TGTCTTACCT	GCTCCAGCAA	GACCTCCTCA	AGAGAACCAC	TCAAGAATGC	600
601	TTCCAGAAAA	CTTTGGTTCT	GTAAGAAGTC	AGCATTCTTC	ATCCAGTAAG	650
651	TTCATACCGG	GGGTCCCCAC	CTGCTTTGAG	GTGAAACCTG	AAGAAAACTC	700
701	GGATGTTCAT	GACATGAGAG	TAAACCGTTC	TATCATGAGA	ATCAAAAAGA	750
751	GATCCCGAAG	TGTTTTCTAT	AGACTAACCA	TACTGATACT	AGTGTTTGCC	800
801	GTTAGCTGGA	TGCCACTACA	CCTTTTCCAT	GTGGTAACTG	ATTTTAATGA	850
851	CAACCTCATT	TCAAACAGGC	ATTTCAAATT	GGTGTATTGC	ATTTGTCATT	900
901	TGTTAGGCAT	GATGTCCTGT	TGTCTTAATC	CTATTCTGTA	TGGTTTTCTC	950
951	AATAATGGGA	TCAAAGCTGA	TTTAATTTCC	CTTATACAGT	GTCTTCATAT	1000
1001	GTCATAATTA	TTAATGTTTA	CCAAGGAGAC	AACAAATGTT	GGGATCGTCT	1050
1051	AAAA					

FIGURE 15

```
  1  MCHIMPFLQC VSVLVSTLIL ISIAIVRYHM IKHPISNNLT ANHGYFLIAT   50
 51  VWTLGFAICS PLPVFHSLVE LQETFDSALL SSRYLCVESW PSDSYRIAFT  100
101  ISLLLVQYIL PLVCLTVSHT SVCRSISCGL SNKENKLEEN EMINLTLQPF  150
151  KKSGPQVKLS SSHKWSYSFI RKHRRRYSKK TACVLPAPAR PPQENHSRML  200
201  PENFGSVRSQ HSSSSKFIPG VPTCFEVKPE ENSDVHDMRV NRSIMRIKKR  250
251  SRSVFYRLTI LILVFAVSWM PLHLFHVVTD FNDNLISNRH FKLVYCICHL  300
301  LGMMSCCLNP ILYGFLNNGI KADLISLIQC LHMS
```

FIGURE 16A

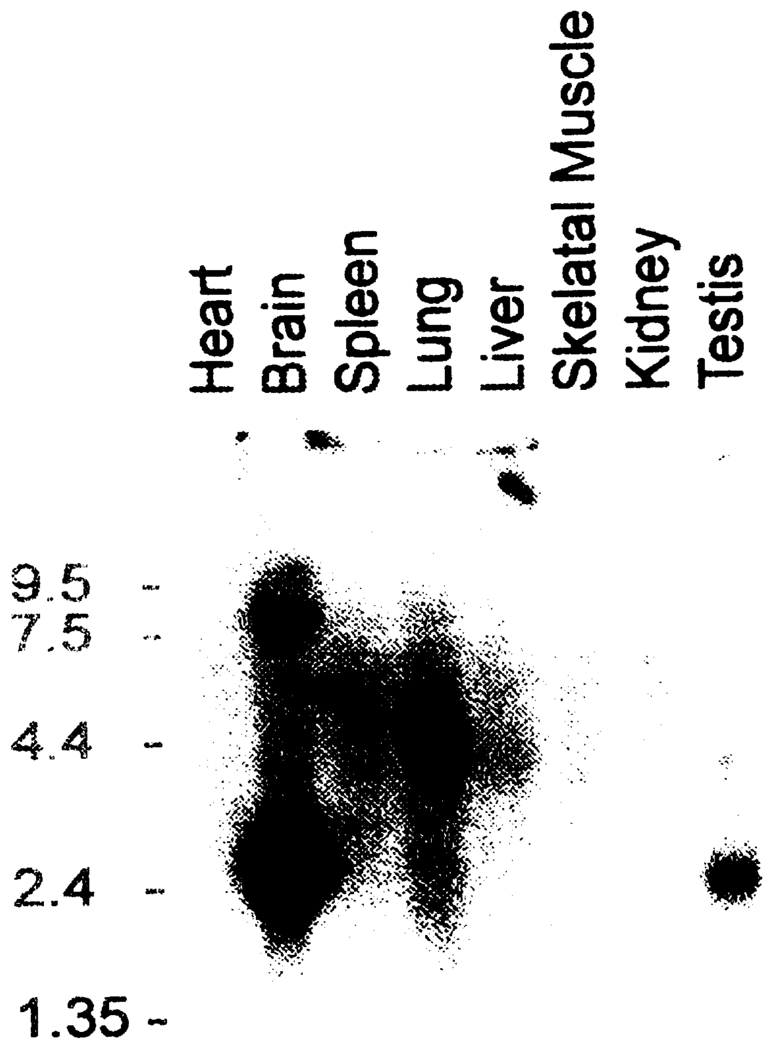

Heart
Brain
Spleen
Lung
Liver
Skelatal Muscle
Kidney
Testis
9.5
7.5
4.4
2.4
1.35

FIGURE 16B

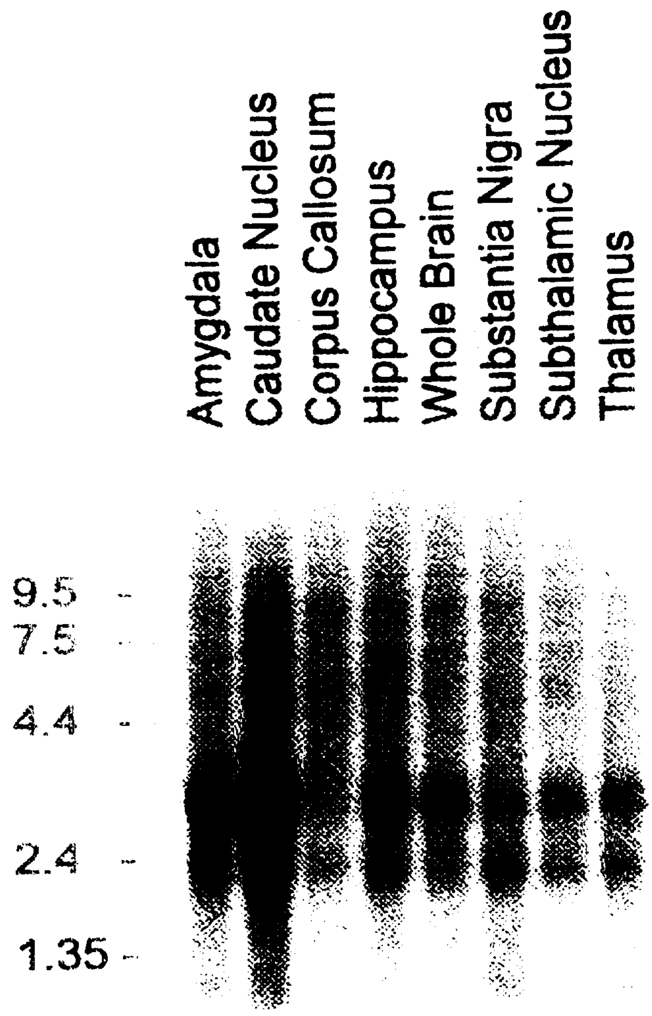

Amygdala
Caudate Nucleus
Corpus Callosum
Hippocampus
Whole Brain
Substantia Nigra
Subthalamic Nucleus
Thalamus
9.5
7.5
4.4
2.4
1.35

FIGURE 16C

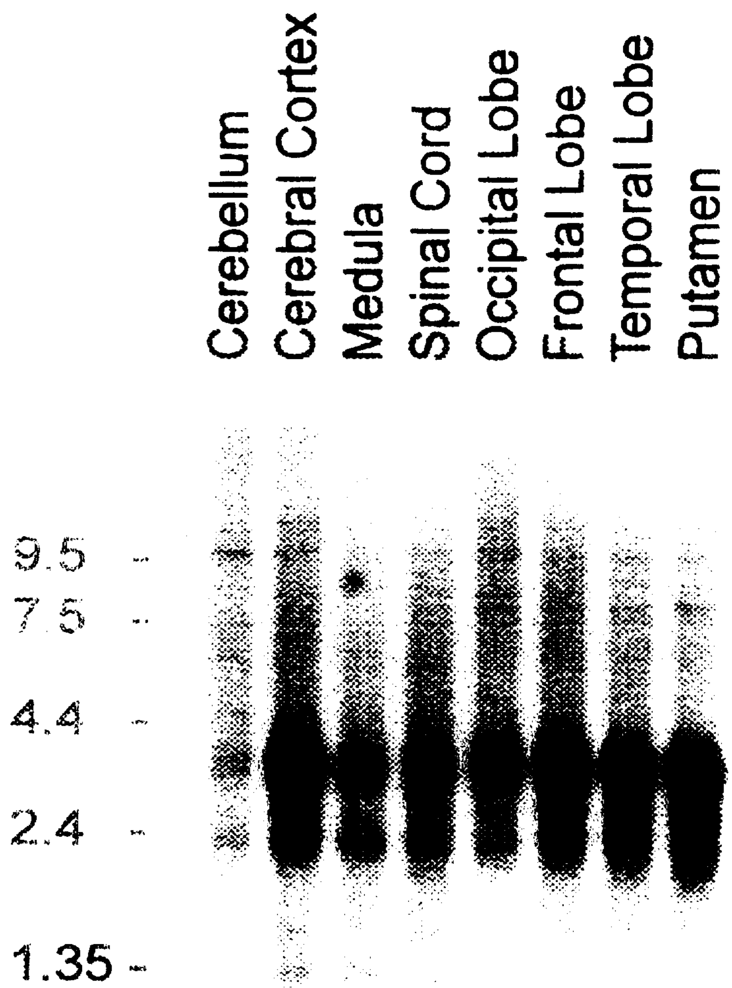

Cerebellum
Cerebral Cortex
Medula
Spinal Cord
Occipital Lobe
Frontal Lobe
Temporal Lobe
Putamen
9.5
7.5
4.4
2.4
1.35

FIGURE 17A

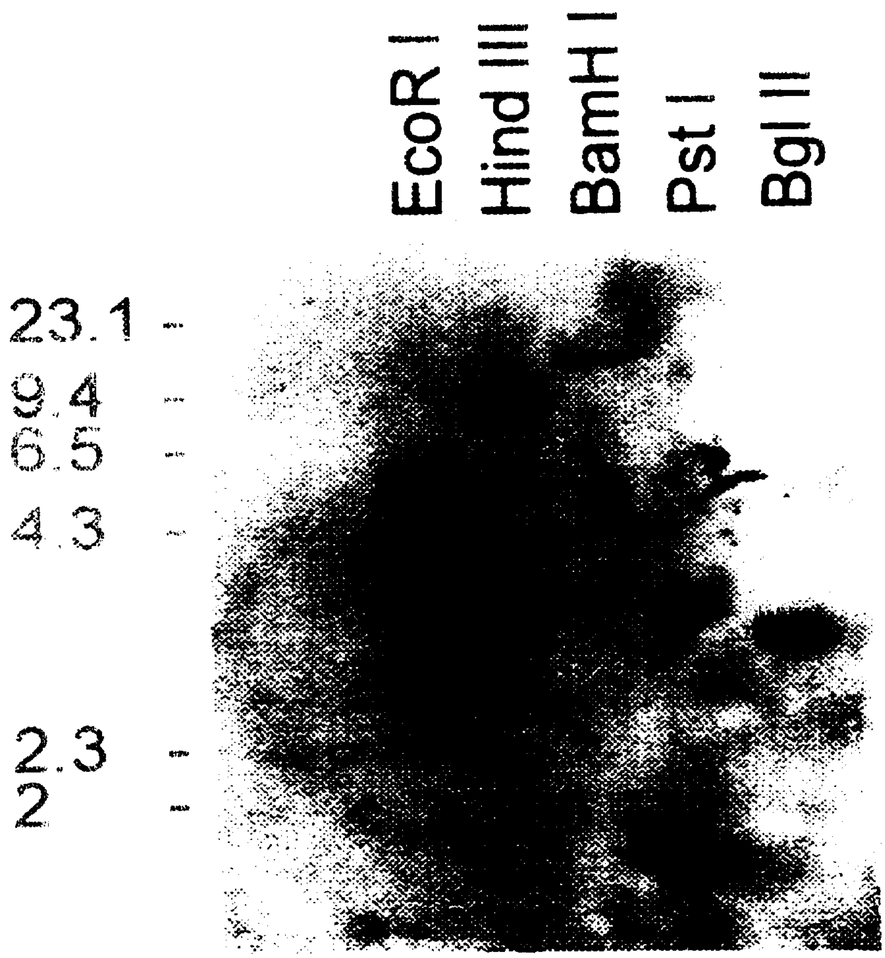


FIGURE 17B

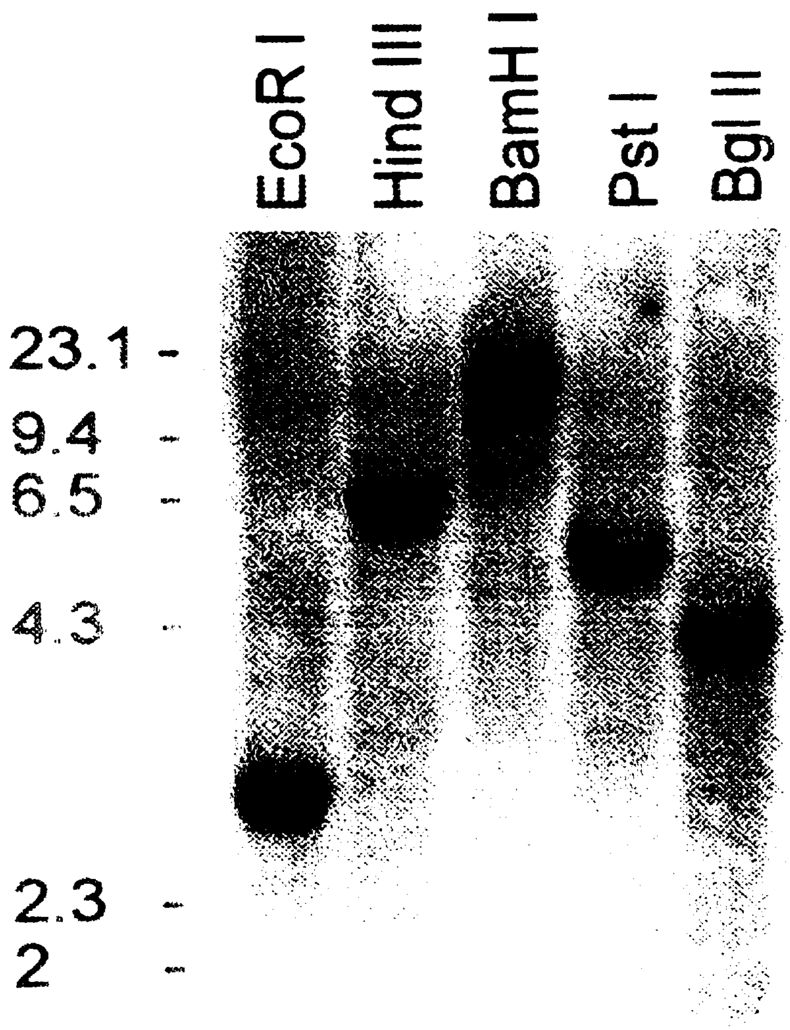


FIGURE 18

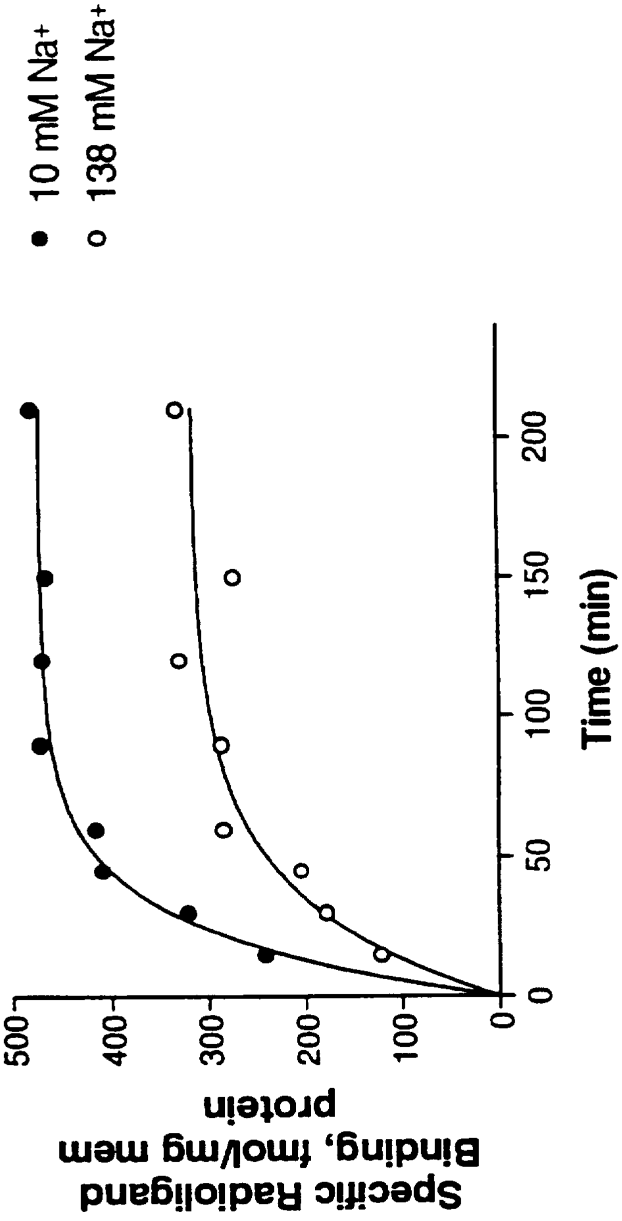

10 mM Na+
138 mM Na+
Specific Radioligand Binding, fmol/mg mem protein
0
100
200
300
400
500
0
50
100
150
200
Time (min)

FIGURE 19

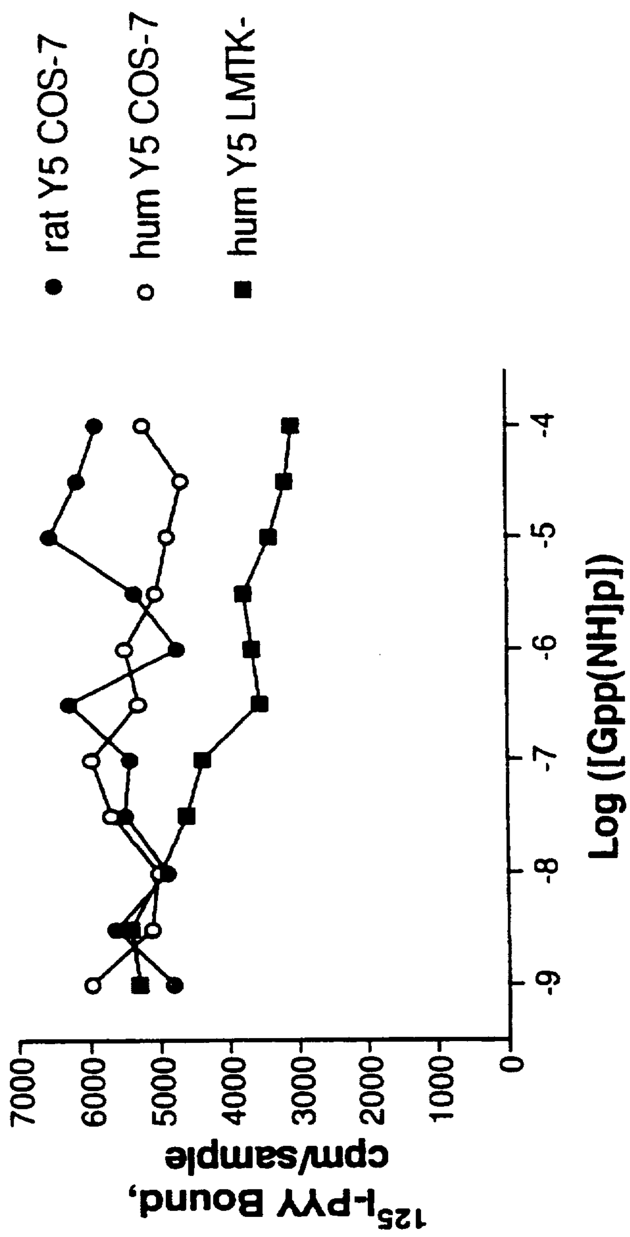

rat Y5 COS-7
hum Y5 COS-7
hum Y5 LMTK-
^{125}I-PYY Bound, cpm/sample
7000
6000
5000
4000
3000
2000
1000
0
-9
-8
-7
-6
-5
-4
Log ([Gpp(NH)p])

FIGURE 20

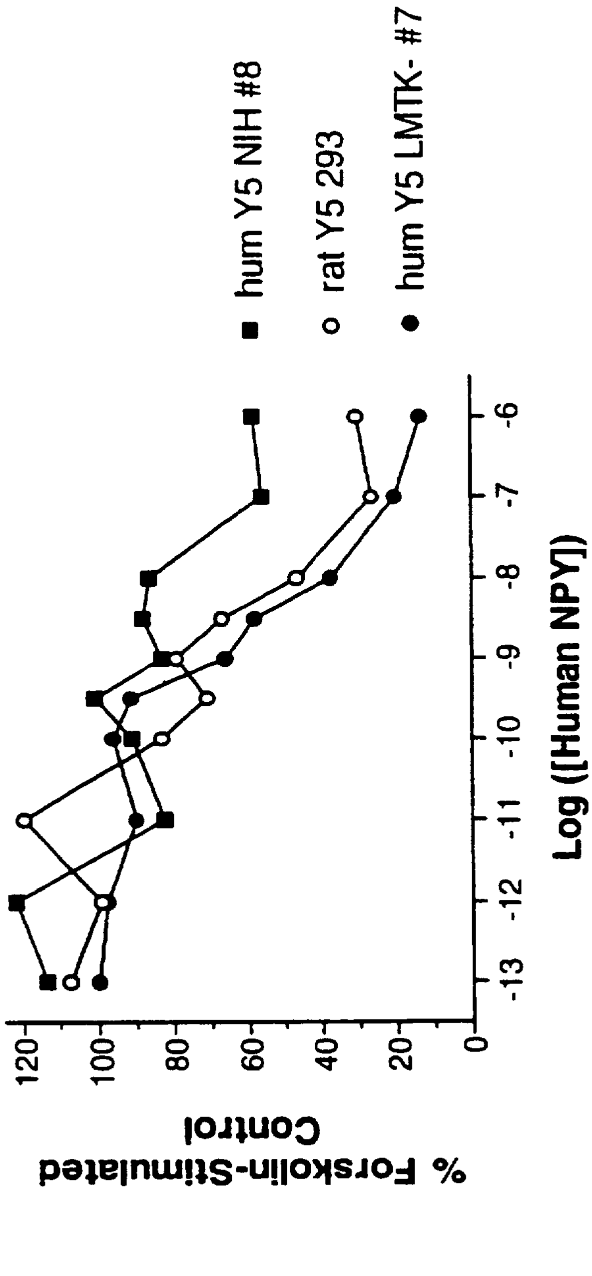

% Forskolin-Stimulated Control
120
100
80
60
40
20
0
-13
-12
-11
-10
-9
-8
-7
-6
Log ([Human NPY])
hum Y5 NIH #8
rat Y5 293
hum Y5 LMTK- #7

FIGURE 21A

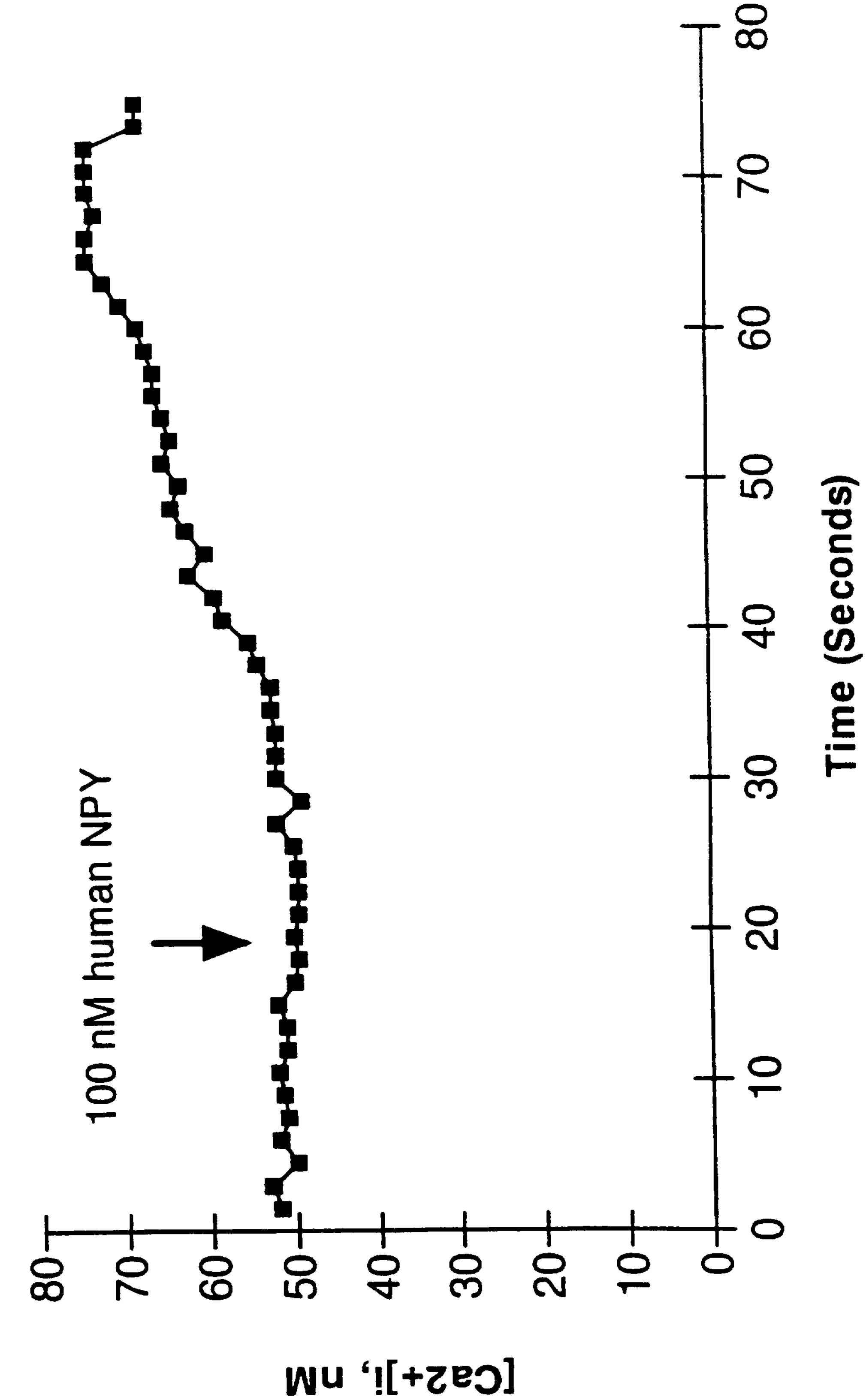

100 nM human NPY
[Ca2+]i, nM
0
10
20
30
40
50
60
70
80
Time (Seconds)
0
10
20
30
40
50
60
70
80

FIGURE 21B

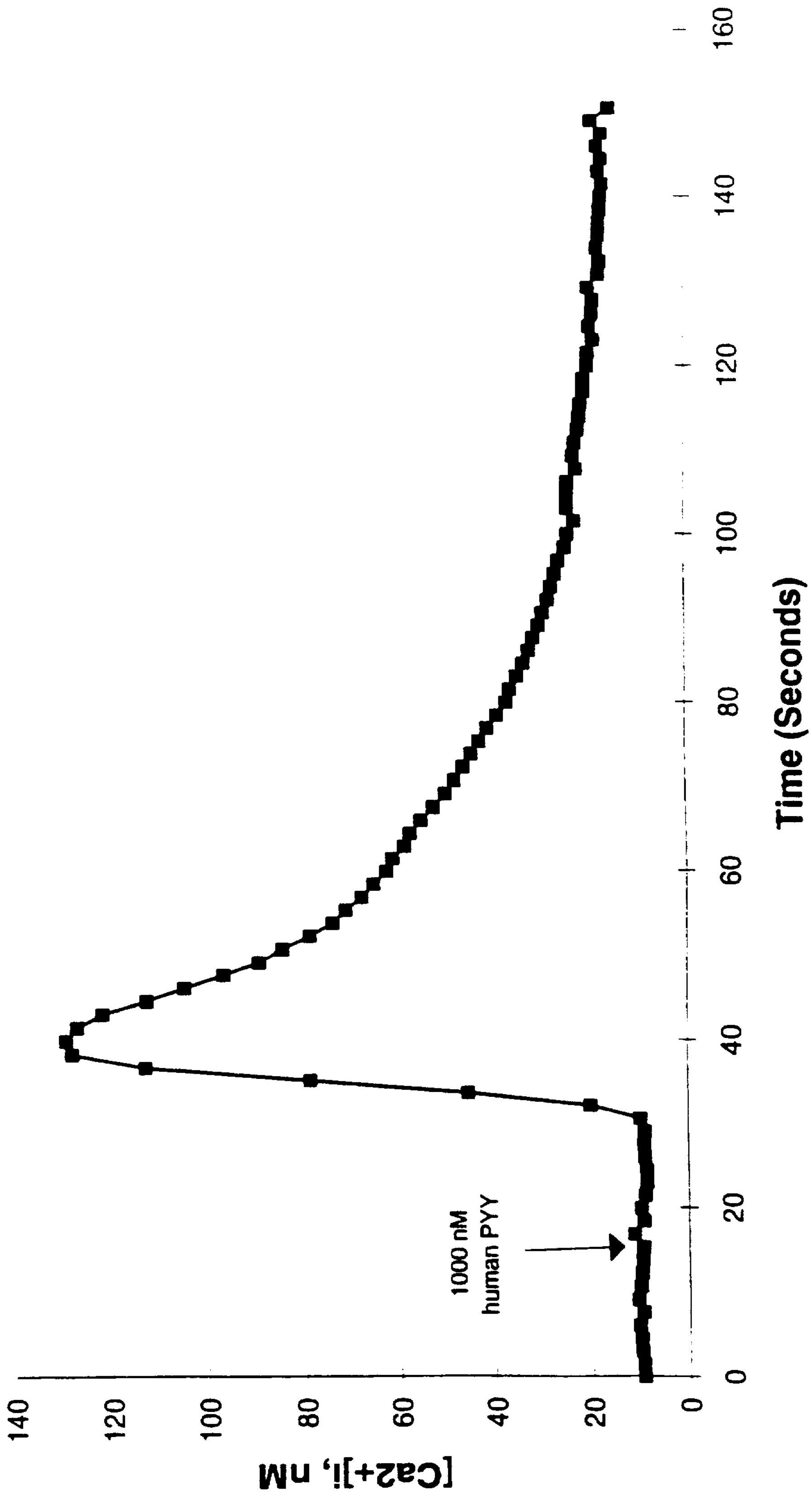

[Ca2+]i, nM
140
120
100
80
60
40
20
0
Time (Seconds)
0
20
40
60
80
100
120
140
160
1000 nM
human PYY

FIGURE 21C

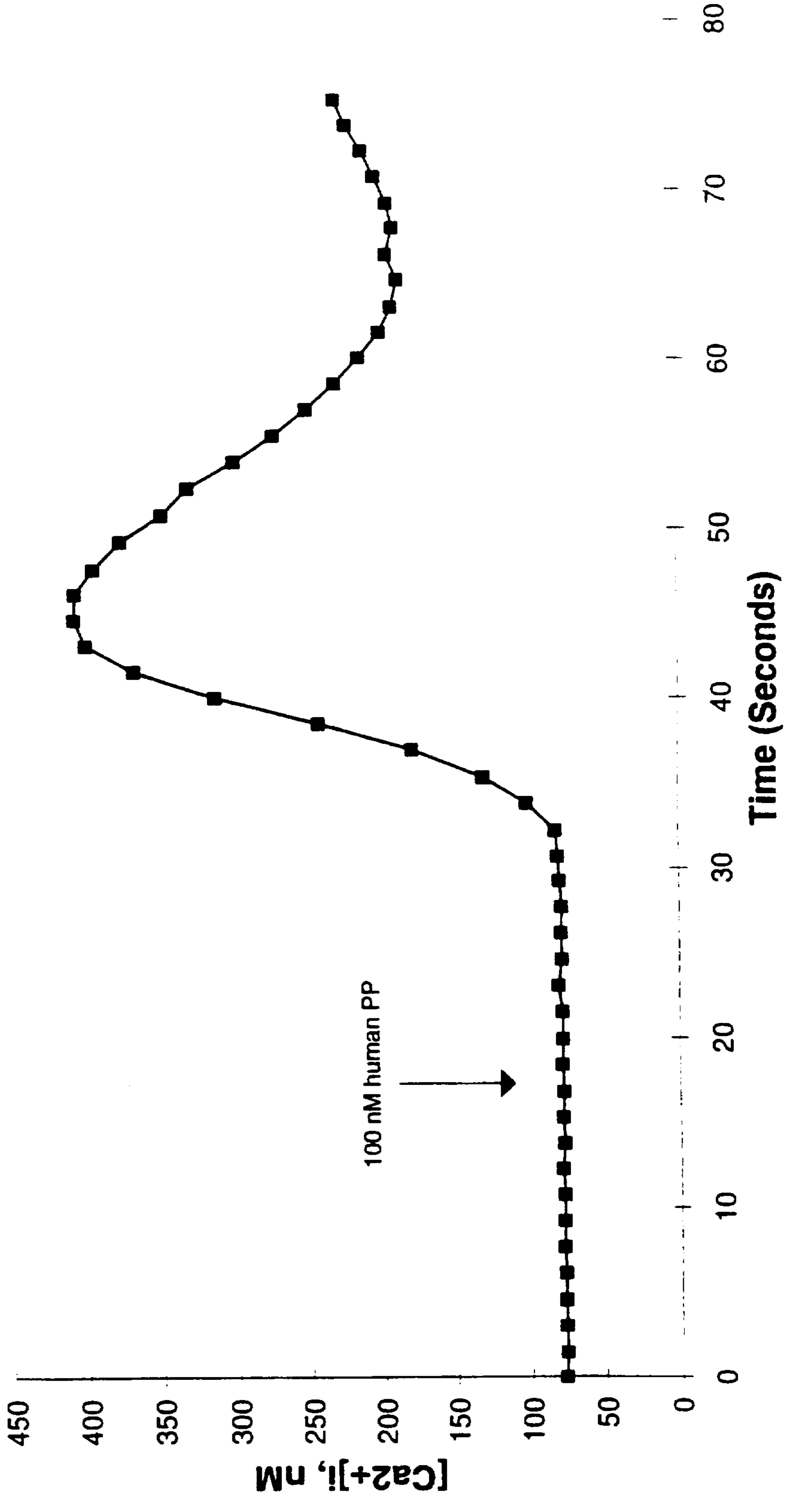

100 nM human PP
[Ca2+]i, nM
450
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
Time (Seconds)

FIGURE 21D

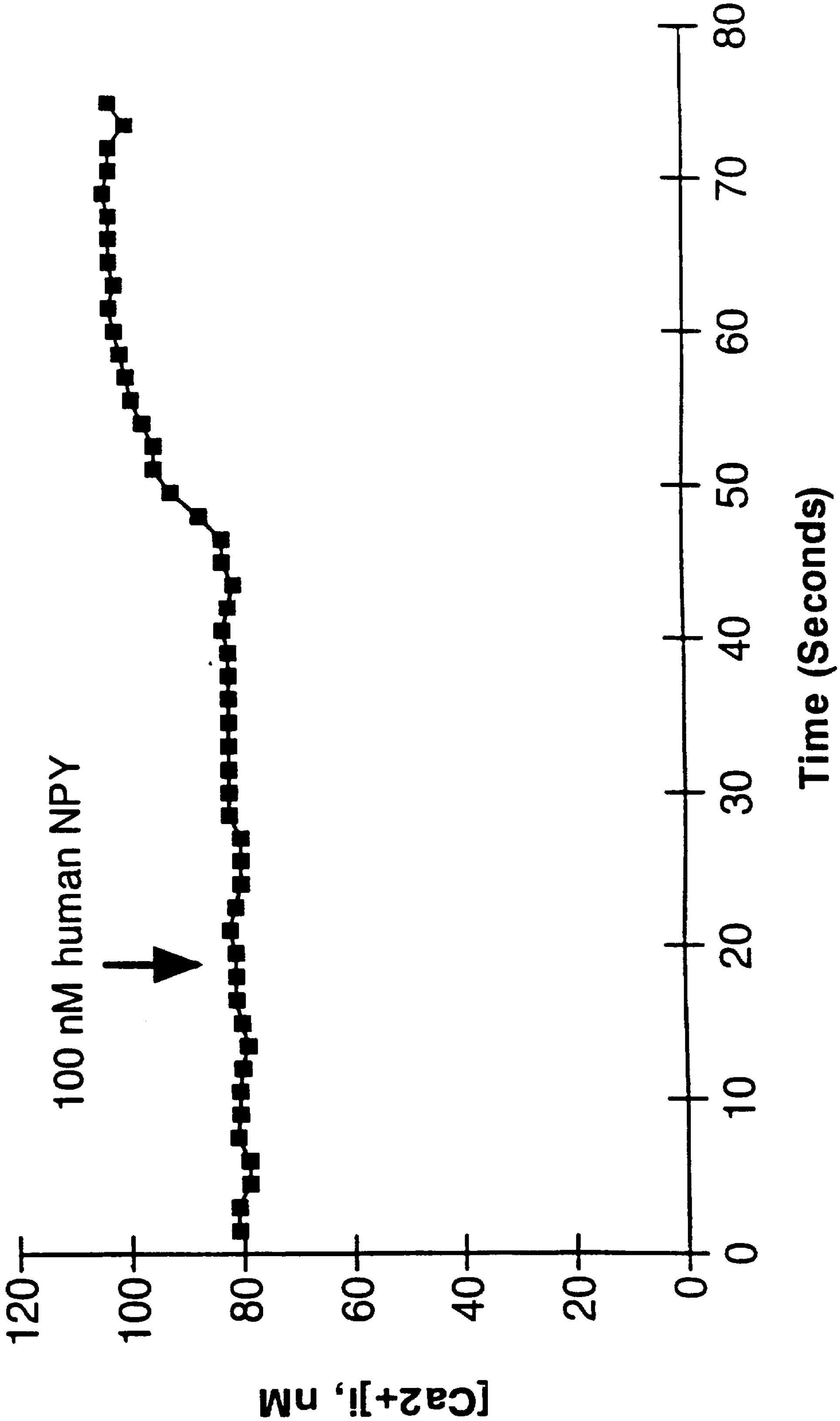
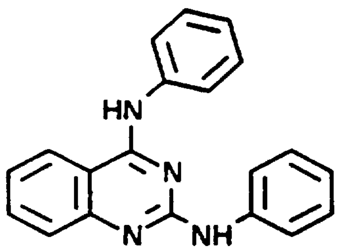
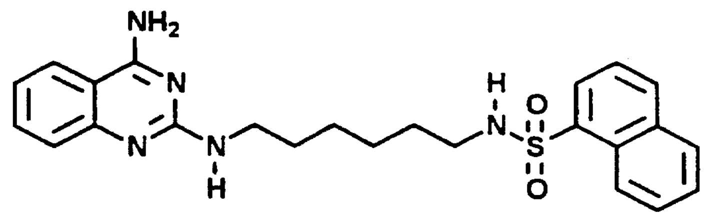

100 nM human NPY
[Ca2+]i, nM
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80
Time (Seconds)

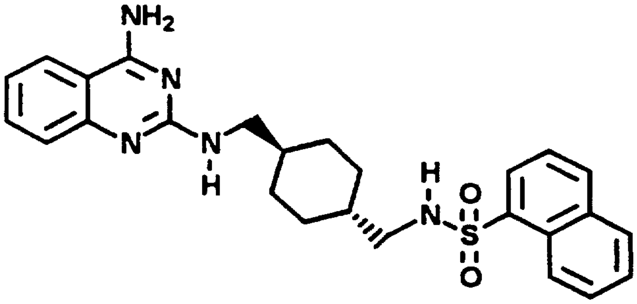
FIGURE 22A



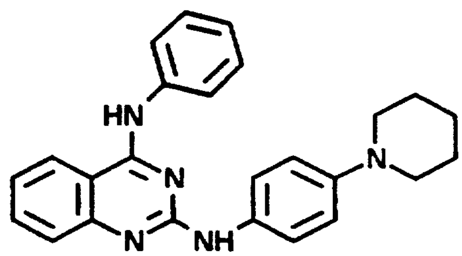
Compound 1



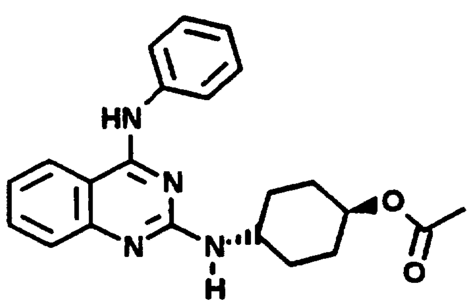
Compound 2



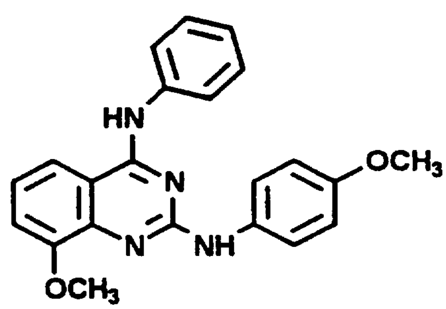
Compound 5



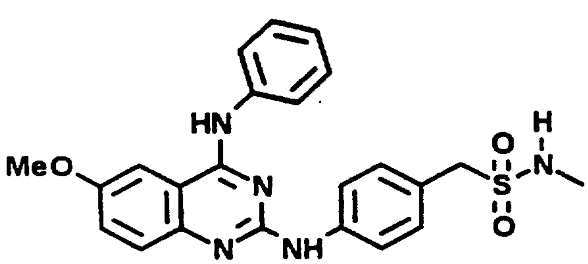
Compound 6



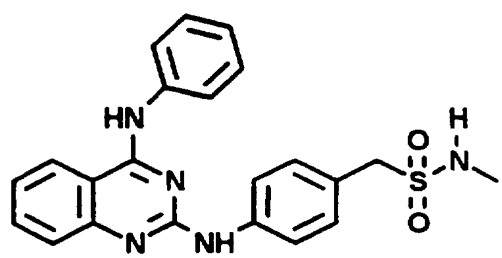
Compound 7



Compound 9

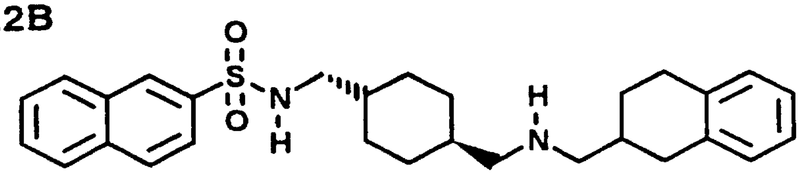


Compound 10

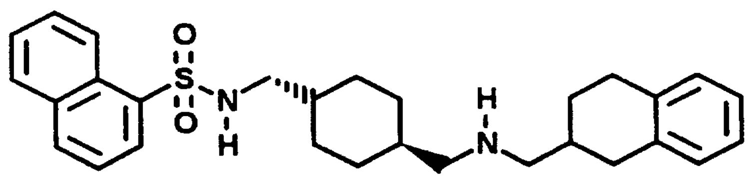


Compound 11

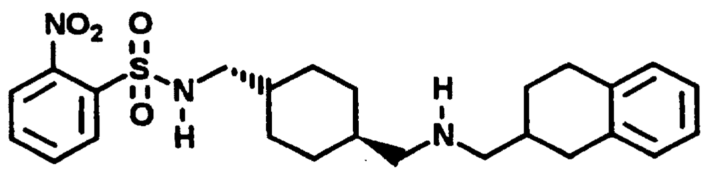
FIGURE 22B



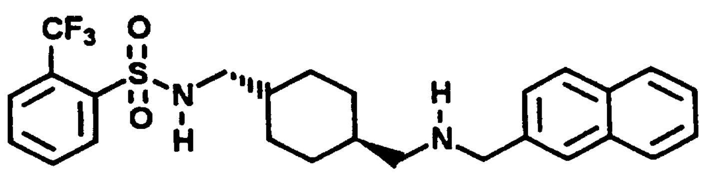
Compound 17



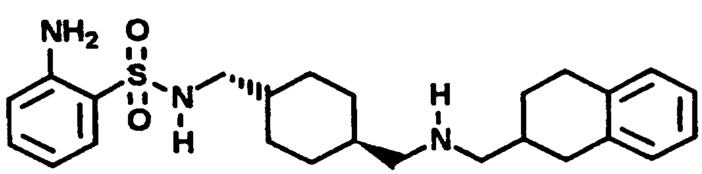
Compound 19



Compound 20

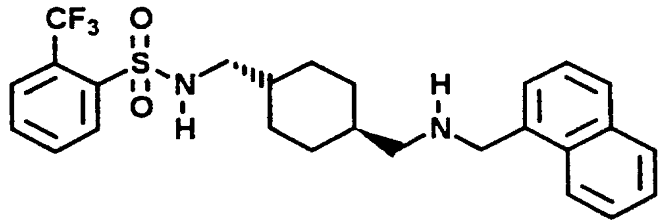


Compound 21

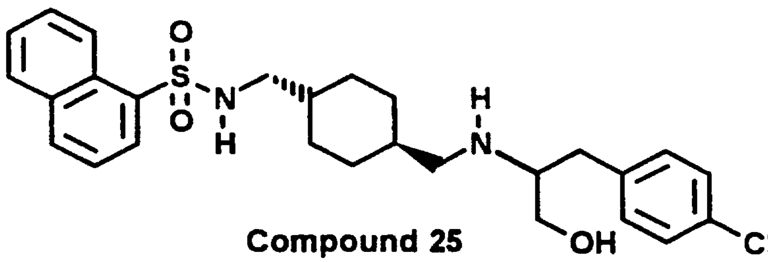


Compound 22

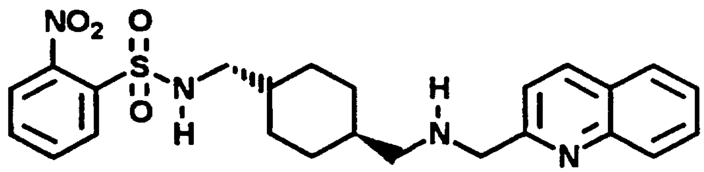
FIGURE 22C



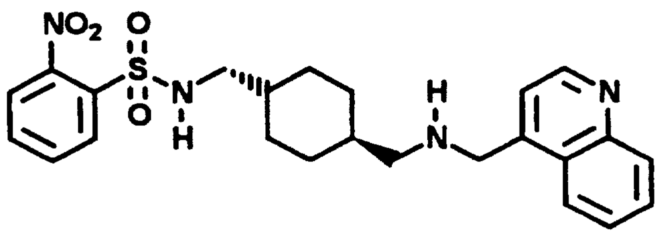
Compound 23



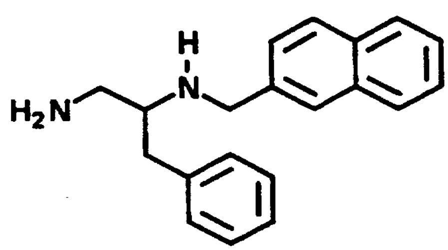
Compound 25



Compound 26



Compound 27



Compound 28

FIGURE 23

```
   1 GTAGTCTCCCTCTCAGAATTGATTTATCGTAGTCATGTAATTTTTAAAAGTTGGTAACT   60
  61 AATGTCTTTTTATTCCAAGCAGAACTCTAAGATGGATTTAGAACTCCAGGATTTTTATAA  120
 121 CAAGACACTTGCCACAGAGAACAATACGGCTGCCACTCGGAATTCTGATTTCCCAGTCTG  180
 181 GGATGACTATAAAAGCAGTGTAGATGATTTACAGTATTTTCTGATTGGACTTTATACATT  240
 241 TGTAAGTCTTCTCGGTTTTATGGGGAATCTACTTATTTTAATGGCTCTCATGAGAAAGCG  300
 301 TAATCAGAAGACGATGGTAAACTTCCTCATAGGAAATTTGGCCTTCTCTGATATTTTGGT  360
 361 TGTGCTGTTTTGCTCACCTTTTACACTGACCTCTGTCCTGCTGGATCAGTGGATGTTTGG  420
 421 CAAAGTCATGTGTCACATTATGCCTTTTCTTCAATGTGTGTCAGTTCTGGTTTCAACTTT  480
 481 AATTCTAATATCAATTGCCATTGTCAGGTATCATATGATCAAGCATCCTATATCTAATAA  540
 541 TTTAACAGCAAACCATGGCTACTTCCTGATTGCTACTGTCTGGACACTAGGTTTTGCGAT  600
 601 TTGTTCTCCCCTTCCAGTGTTTCACAGTCTGGTGGAACTTCAGGAAACATTTGACTCCGC  660
 661 ATTGCTGAGCAGCAGGTATTTATGTGTTGAGTCGTGGCCATCTGATTCGTACAGAATCGC  720
 721 TTTTACTATCTCTTTATTGCTAGTCCAGTATATTCTTCCCTTGGTGTGTCTAACTGTGAG  780
 781 CCATACCAGTGTCTGCAGGAGTATAAGCTGCGGGTTGTCCAACAAAGAAAACAAACTGGA  840
 841 AGAAAACGAGATGATCAACTTAACTCTTCAACCATTCAAAAAGAGTGGGCCTCAGGTGAA  900
 901 ACTTTCCAGCAGCCATAAATGGAGCTATTCATTCATCAGAAAACACAGGAGAAGGTACAG  960
 961 CAAGAAGACGGCGTGTGTCTTACCTGCTCCAGCAAGACCTCCTCAAGAGAACCACTCAAG 1020
1021 AATGCTTCCAGAAAACTTTGGTTCTGTAAGAAGTCAGCATTCTTCATCCAGTAAGTTCAT 1080
1081 ACCGGGGGTCCCCACCTGCTTTGAGGTGAAACCTGAAGAAAACTCGGATGTTCATGACAT 1140
1141 GAGAGTAAACCGTTCTATCATGAGAATCAAAAAGAGATCCCGAAGTGTTTTCTATAGACT 1200
1201 AACCATACTGATACTAGTGTTTGCCGTTAGCTGGATGCCACTACACCTTTTCCATGTGGT 1260
1261 AACTGATTTTAATGACAACCTCATTTCAAACAGGCATTTCAAATTGGTGTATTGCATTTG 1320
1321 TCATTTGTTAGGCATGATGTCCTGTTGTCTTAATCCTATTCTGTATGGTTTTCTCAATAA 1380
1381 TGGGATCAAAGCTGATTTAATTTCCCTTATACAGTGTCTTCATATGTCATAATTCTTCAT 1440
1441 GTTTACCAAGGAGACAACAAATGTTGGGATCGTCTAAAA                      1480
```

FIGURE 24

1	M	S	F	Y	S	K	Q	N	S	K	M	D	L	E	L	Q	D	F	Y	N	20
21	K	T	L	A	T	E	N	N	T	A	A	T	R	N	S	D	F	P	V	W	40
41	D	D	Y	K	S	S	V	D	D	L	Q	Y	F	L	I	G	L	Y	T	F	60
61	V	S	L	L	G	F	M	G	N	L	L	I	L	M	A	L	M	R	K	R	80
81	N	Q	K	T	M	V	N	F	L	I	G	N	L	A	F	S	D	I	L	V	100
101	V	L	F	C	S	P	F	T	L	T	S	V	L	L	D	Q	W	M	F	G	120
121	K	V	M	C	H	I	M	P	F	L	Q	C	V	S	V	L	V	S	T	L	140
141	I	L	I	S	I	A	I	V	R	Y	H	M	I	K	H	P	I	S	N	N	160
161	L	T	A	N	H	G	Y	F	L	I	A	T	V	W	T	L	G	F	A	I	180
181	C	S	P	L	P	V	F	H	S	L	V	E	L	Q	E	T	F	D	S	A	200
201	L	L	S	S	R	Y	L	C	V	E	S	W	P	S	D	S	Y	R	I	A	220
221	F	T	I	S	L	L	L	V	Q	Y	I	L	P	L	V	C	L	T	V	S	240
241	H	T	S	V	C	R	S	I	S	C	G	L	S	N	K	E	N	K	L	E	260
261	E	N	E	M	I	N	L	T	L	Q	P	F	K	K	S	G	P	Q	V	K	280
281	L	S	S	S	H	K	W	S	Y	S	F	I	R	K	H	R	R	R	Y	S	300
301	K	K	T	A	C	V	L	P	A	P	A	R	P	P	Q	E	N	H	S	R	320
321	M	L	P	E	N	F	G	S	V	R	S	Q	H	S	S	S	S	K	F	I	340
341	P	G	V	P	T	C	F	E	V	K	P	E	E	N	S	D	V	H	D	M	360
361	R	V	N	R	S	I	M	R	I	K	K	R	S	R	S	V	F	Y	R	L	380
381	T	I	L	I	L	V	F	A	V	S	W	M	P	L	H	L	F	H	V	V	400
401	T	D	F	N	D	N	L	I	S	N	R	H	F	K	L	V	Y	C	I	C	420
421	H	L	L	G	M	M	S	C	C	L	N	P	I	L	Y	G	F	L	N	N	440
441	G	I	K	A	D	L	I	S	L	I	Q	C	L	H	M	S					456

Any reference to figure 25 shall be considered non-existent (See Article 14(2))

INTERNATIONAL SEARCH REPORT	International application No. PCT/US97/09504

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ---- A, P	US 5,602,024 A (GERALD ET AL.) 11 FEBRUARY 1997, COLUMN 6, LINE 43 TO COLUMN 16, LINE 36.	1-3, 5-14, 16-33, 38-63, 65, 67-142 ------ 4, 15, 34-37, 64, 66, 143-155
Y	WAHLESTEDT ET AL. IDENTIFICATION OF CULTURED CELLS SELECTIVELY EXPRESSING Y1-, Y2-, OR Y3-TYPE RECEPTORS FOR NEUROPEPTIDE Y/PEPTIDE YY. LIFE SCIENCES. 1991, VOLUME 50, PAGES PL-7 - PL-12, ESPECIALLY PAGES PL-10 - PL-11.	45-61, 69-72, 76-83, 98-120, 137-142

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date		
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 SEPTEMBER 1997	17 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer STEPHEN GUCKER Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HERZOG et al. Cloned human neuropeptide Y receptor couples to two different second messenger systems. Proc. Natl. Acad. Sci. USA. July 1992, Volume 89, pages 5794-5798, especially pages 5794-5798.	45-61, 69-72, 76-83, 98-120, 137-142
Y	GEHLERT, D. R. Subtypes of receptors for neuropeptide Y: implications for the targeting of therapeutics. Life Sciences. 1994, Volume 55, pages 551-562, especially pages 552 and 556-558.	45-61, 69-72, 76-83, 98-120, 137-142

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09504

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09504

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/02, 39/395, 48/00; C07H 21/02, 21/04; C07K 14/00, 14/435, 14/705, 16/00; C12N 15/00, 15/12, 15/63; C12P 21/06; G01N 33/53, 33/566, 33/567

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/178.1; 435/7.21, 69.1, 70.1, 320.1, 325, 348, 357, 358, 365, 369; 514/2, 44, 909, 964; 530/300, 350, 387.9, 388.1; 536/23.1, 23.5, 24.1, 24.31; 800/2

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/178.1; 435/7.21, 69.1, 70.1, 320.1, 325, 348, 357, 358, 365, 369; 514/2, 44, 909, 964; 530/300, 350, 387.9, 388.1; 536/23.1, 23.5, 24.1, 24.31; 800/2

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, SCISEARCH, EMBASE, BIOSIS, CAPLUS, WPIDS, BIOTECHDS, DISSABS, CONFSCI, LIFESCI

search terms: neuropeptide#, NPY, receptor#, cAMP, adenylate, calcium, binding, feeding, antibod###

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-25, 45-61, and 85-86, drawn to nucleic acids, probes, vectors, host cells, encoded protein, method of making encoded protein, and a method to use the encoded protein in an assay.

Group II, claim(s) 26-28 and 33-37, drawn to functional antisense nucleotides.

Group III, claim(s) 29-32, 38, and 73, drawn to antibodies.

Group IV, claims 39-44, drawn to transgenic animals.

Group V, claims 62-64, drawn to agonists.

Group VI, claims 65-67, drawn to antagonists.

Group VII, claim 68, drawn to a detection method using hybridization.

Group VIII, claims 69-70, drawn to a therapeutic method using antisense nucleotides.

Group IX, claims 71-72, 80 and 83, drawn to a therapeutic method of using agonists.

Group X, claims 74-75, drawn to an investigative method of using transgenic animals.

Group XI, claims 76, 79, and 98-120, drawn to a method of administering antagonists for therapeutic effects.

Group XII, claims 77-78, drawn to therapeutically effective antagonists.

Group XIII, claims 81-82, drawn to therapeutically effective agonists.

Group XIV, claims 84 and 87-97, drawn to a diagnostic method.

Group XV, claims 121-136, drawn to therapeutic methods using a combination of agonists and monoamine neurotransmitter uptake inhibitors.

Group XVI, claims 137-142, drawn to methods involving guanine nucleotide binding.

Group XVII, claims 143-155, drawn to therapeutic methods using a combination of galanin and Y5 receptor antagonists.

The inventions listed as Groups I-XVII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group II is drawn to functional antisense nucleotides and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group III is drawn to antibodies and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group IV is drawn to transgenic animals and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group V is drawn to agonists and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group VI is drawn to antagonists and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group VII is drawn to a detection method using hybridization and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group VIII is drawn to a therapeutic method using antisense nucleotides and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group IX is drawn to a therapeutic method of using agonists and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group X is drawn to an investigative method of using transgenic animals and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group XI is drawn to a method of administering antagonists for therapeutic effects and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group XII is drawn to therapeutically effective antagonists and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group XIII is drawn to therapeutically effective agonists and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group XIV is drawn to a diagnostic method and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group XV is drawn to therapeutic methods using a combination of agonists and monoamine neurotransmitter uptake inhibitors and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group XVI is drawn to methods involving guanine nucleotide binding and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Group XVII is drawn to therapeutic methods using a combination of galanin and Y5 receptor antagonists and lacks the special technical feature of nucleotides encoding the canine Y5 receptor of Group I.

Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to

form a single inventive concept.